

09/24/99

Jc574 U.S. PTO

<b>UTILITY PATENT APPLICATION TRANSMITTAL</b> <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	Attorney Docket No.	B0801/7155
	First Named Inventor or Application Identifier	
	PIER	
	Express Mail Label No.	EL024660127US
	Date of Deposit	September 24, 1999

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<b>APPLICATION ELEMENTS</b> <i>See MPEP chapter 600 concerning utility patent application contents</i>	<b>ADDRESS</b> TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231
<p>1. <input type="checkbox"/> Fee Transmittal Form <i>(Submit an original, and a duplicate for fee processing)</i></p> <p>2. <input checked="" type="checkbox"/> Specification [Total pages 95] 79 pages description 1 page abstract 15 pages claims (#1-68)</p> <p>3. <input type="checkbox"/> Drawing(s) (35 USC 113) <input type="checkbox"/> Informal <input type="checkbox"/> Formal</p> <p>4. <input checked="" type="checkbox"/> Oath or Declaration [Total pages ] a. <input type="checkbox"/> Newly executed (original or copy) b. <input checked="" type="checkbox"/> Copy from a prior application (37 CFR 1.63(d)) <i>(for continuation/divisional with Box 17 completed)</i> [Note Box 5 below] i. <input type="checkbox"/> <b>DELETION OF INVENTOR(S)</b> Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</p> <p>5. <input checked="" type="checkbox"/> Incorporation by Reference <i>(usable if Box 4b is checked)</i> The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.</p>	<p>6. <input type="checkbox"/> Microfiche Computer Program (Appendix)</p> <p>7. <input type="checkbox"/> Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. <input type="checkbox"/> Computer Readable Copy b. <input type="checkbox"/> Paper Copy (identical to computer copy) c. <input type="checkbox"/> Statement verifying identity of above copies</p> <p><b>ACCOMPANYING APPLICATION PARTS</b></p> <p>8. <input type="checkbox"/> Assignment Papers (cover sheet &amp; documents(s))</p> <p>9. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney <i>(when there is an assignee)</i></p> <p>10. <input type="checkbox"/> English Translation of Document <i>(if applicable)</i></p> <p>11. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations</p> <p>12. <input checked="" type="checkbox"/> Preliminary Amendment</p> <p>13. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) <i>(Should be specifically itemized)</i></p> <p>14. <input type="checkbox"/> Small Entity <input checked="" type="checkbox"/> Statement filed in prior application, Status still proper and desired</p> <p>15. <input type="checkbox"/> Certified Copy of Priority Document(s) <i>(if foreign priority is claimed)</i></p>
16. Other: This application is a Divisional Application of US Application Serial No. 08/681,838 filed July 29, 1996, pending.	

**17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:**

☐ Continuation    ☒ Divisional    ☐ Continuation-in-part

☐ Cancel in this application claims before calculating the filing fee.


☐ Amend the specification by inserting before the first line the sentence:

**18. CORRESPONDENCE ADDRESS**

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**19. SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED**

<b>NAME</b>	Helen C. Lockhart, Reg. No. 39,248
<b>SIGNATURE</b>	
<b>DATE</b>	September 24, 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gerald B. Pier  
Filed: Herewith  
METHODS AND PRODUCTS FOR TREATING  
PSEUDOMONAS INFECTION  
Examiner: Unassigned  
Art Unit: 1646

THE ASSISTANT COMMISSIONER  
FOR PATENTS  
WASHINGTON, D.C. 20231

Sir:

**PRELIMINARY AMENDMENT**

Please amend the Application as follows:

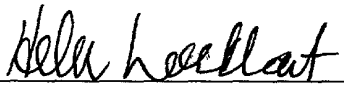
Before calculating fees, please cancel claims 62-68.

Please insert on page 1, before Background of the Invention, the following:

**---Related Applications**

This application is a Divisional Application of US Application Serial No.  
08/681,838 filed July 29, 1996, now pending, which is incorporated by reference.---

Respectfully submitted,

  
\_\_\_\_\_  
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Docket No.B0801/7155  
September 24, 1999  
**XNDD**

Express Mail Label No. EL024660127US  
Date of Deposit: September 24, 1999

**Application**  
**For**  
**United States Letters Patent**  
**by**  
**Gerald B. Pier**

**For**

**METHODS AND PRODUCTS FOR**  
**TREATING PSEUDOMONAS INFECTION**

## **Background of the Invention**

Cystic fibrosis (CF) is a disease that arises due to mutations in the gene that codes for cystic fibrosis transmembrane conductance regulator (CFTR), which is a membrane protein involved in chloride ion secretion [1]. Although most cystic fibrosis patients develop chronic progressive disease of the respiratory system, the disease can cause damage to many other organs and tissues. For instance, pancreatic dysfunction, hepatobiliary and genitourinary diseases are all common manifestations of the cystic fibrosis disorder. The diverse array of symptoms and disorders caused by cystic fibrosis have made treatment of the disorder a difficult task. Many treatment modes have focused on improving the clinical symptoms of the particular organ affected in the patient, such as antibiotic treatments, improved nutritional care, and physiotherapy. Additionally, therapies have been developed which attempt to counteract the biochemical basis of the genetic disease, such as gene therapy with CFTR genes. None of these treatment methods, however, has been entirely successful in the treatment of cystic fibrosis.

The most serious consequence of cystic fibrosis (CF) is *Pseudomonas aeruginosa* lung infection, which by itself accounts for almost 90% of the morbidity and mortality in CF [3]. By age 12, 60-90% of CF patients are infected with *P. aeruginosa*, and most die before age 30 [3]. Pathogens such as *S. aureus* and nontypable *H. influenzae* are also commonly isolated from the respiratory tract of CF patients, but only *P. aeruginosa* infection has been associated with the progressive decline in pulmonary function in these patients [4-6].

Progressive loss of pulmonary function over many years due to chronic infection with mucoid *P. aeruginosa* is the hallmark of CF, and yet the connection between lung infection and defects in chloride ion conductance have remained elusive. Smith et al. [2] recently reported defective bacterial killing by fluid obtained from airway epithelial cell cultures of CF patients. Smith et al. reported that this phenomenon was due to the inhibition of an unidentified antimicrobial factor resulting from increased levels of sodium chloride in the airway epithelial fluid.

Many of the severe cases of CF are associated with CFTR mutations leading to greatly reduced to no cell-surface expression of CFTR. The most prevalent of the CFTR mutations is

the deletion of phenylalanine 508. Mutant CFTR genes having a deleted phenylalanine 508 are referred to as  $\Delta F508$ .  $\Delta F508$  accounts for approximately 70% of the cystic fibrosis alleles. The  $\Delta F508$  mutation has been associated with elevated sweat chloride levels and severe physiological effects such as chronic pulmonary disease in many patients.

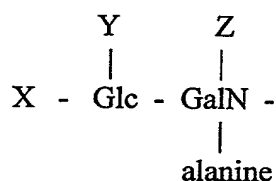
Pier et al. has proposed that ingestion and clearance of *P. aeruginosa* by epithelial cells could be one mechanism by which the epithelial cells protect the lungs against infection [7]. The study reported that ingestion and clearance of *P. aeruginosa* was compromised in a cell line derived from a patient with the  $\Delta F508$  CFTR mutation and was specific for *P. aeruginosa* among the respiratory pathogens evaluated [7]. Expression of wild-type CFTR by transection, or induction of membrane expression of mutant  $\Delta F508$  CFTR by growth of cells at 26°C, increased *P. aeruginosa* ingestion. Inhibition of ingestion of *P. aeruginosa* by cells in neonatal mouse lungs increased the total bacterial load in the lungs [7]. These studies showed that CFTR modulated this epithelial cell process but did not specifically indicate how CFTR was involved in the process.

### Summary of the Invention

The invention involves the discovery that *P. aeruginosa* binds to the cystic fibrosis transmembrane conductance regulator (CFTR) (SEQ.ID.NO.1) and, in particular, that the core portion of the lipopolysaccharide of *P. aeruginosa* binds the CFTR. The invention also involves the discovery that contacting cells expressing the CFTR with the core portion of the lipopolysaccharide of *P. aeruginosa* results in *upregulation* of the CFTR. Upregulation of the CFTR in epithelial mucosa further was discovered to result in better clearance of *P. aeruginosa*, and, therefore, methods for preventing, inhibiting or eradicating *Pseudomonas* infection are provided, including subjects having cystic fibrosis. In general, these discoveries have led to methods and products using fragments of the lipopolysaccharide of *P. aeruginosa* and using fragments of the CFTR in the manufacture of pharmaceutical products, diagnostic products, research tools, and methods relating hereto.

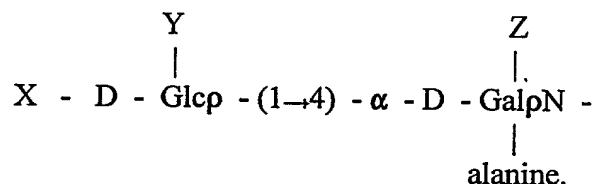
According to one aspect of the invention, a method for upregulating CFTR expression in the tissue of a subject is provided. A CFTR expression regulator is administered to a subject in

need of upregulation of CFTR expression, in an amount effective to increase CFTR expression in the tissue of the subject. The CFTR expression regulator is an isolated polysaccharide that is an LPS core moiety comprising

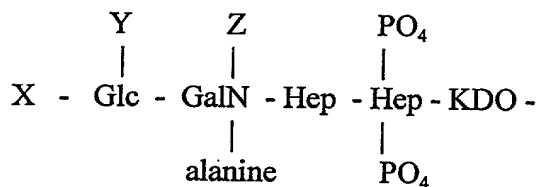


wherein X is selected from the group consisting of glucose, glucose-rhamnose and H; Y is selected from the group consisting of rhamnose and H; and Z is selected from the group consisting of glucose and H.

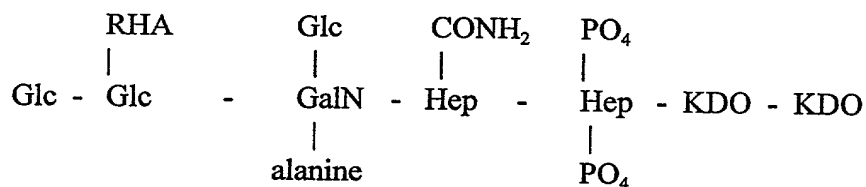
A preferred polysaccharide is an LPS core moiety comprising



One particularly useful polysaccharide according to the invention comprises



Another particularly useful polysaccharide according to the invention is :



The foregoing preferred molecules can be isolated CFTR receptor-binding fragments of lipopolysaccharides of *P. aeruginosa*.

In one embodiment of the invention, the subject has a condition predisposing the subject to *Pseudomonal* infection. In another embodiment of the invention, the subject has a *Pseudomonal* infection. In one important embodiment of the invention, the subject has a defective cystic fibrosis transmembrane conductance regulator gene.

According to another aspect of the invention, a pharmaceutical preparation is provided. The pharmaceutical preparation is a pharmaceutically acceptable carrier and a pharmaceutically effective amount of a CFTR expression regulator. The CFTR expression regulator is as described above. The pharmaceutical preparation can be sterile and can be formulated in a unit dosage in an amount effective for treating *Pseudomonal* infection. As used herein, "treating" means preventing the onset of, slowing the progression of, or eradicating the existence of the condition being treated, such as a *Pseudomonal* infection. The pharmaceutical preparation can be formulated as any suitable preparation, including a preparation suitable for inhalation or a preparation suitable for injection.

According to another aspect of the invention, compositions of matter are provided. The compositions are covalent conjugates. One composition is a covalent conjugate of a lipid biocompatible with a human subject and a polysaccharide. The polysaccharide is as described above. In one embodiment, the lipid portion of the conjugate is inserted within the wall of a liposome and the polysaccharide is exposed on the surface of the liposome. The liposome contains a bioactive agent.

Another composition is a covalent conjugate of a bioactive agent and a polysaccharide. Again, the polysaccharide is as described above.

The foregoing covalent conjugates are useful in delivering bioactive agents to cells and/or tissues expressing a CFTR. Thus, methods are provided for delivering a bioactive agent to a tissue expressing a cystic fibrosis transmembrane conductance regulator to treat a condition susceptible to treatment by the bioactive agent. A bioactive agent coupled to a polysaccharide is administered to a subject in need of such treatment, in an amount effective for treating the



condition. The polysaccharide is as described above. The bioactive agent can be noncovalently or covalently linked to the polysaccharide, or the bioactive agent can be contained in a liposome comprising a lipid biocompatible with a human subject, wherein the polysaccharide is covalently coupled to the lipid.

As a result of the discovery that the CFTR binds the lipopolysaccharide of *P. aeruginosa*, methods and products involving the use of CFTR fragments are provided.

According to one aspect of the invention, a composition of matter is provided. The composition is a covalent conjugate of an anti-*Pseudomonas* drug and CFTR or a lipopolysaccharide-binding fragment of a cystic fibrosis transmembrane conductance regulator. The lipopolysaccharide-binding fragment of a CFTR preferably comprises at least four consecutive amino acids of Sequence ID No. 3, and can comprise at least five, six, seven or eight consecutive amino acids of Sequence ID. No. 3.

According to another aspect of the invention, an isolated polypeptide is provided. The isolated polypeptide is a lipopolysaccharide-binding fragment of a cystic fibrosis transmembrane conductance regulator. The fragment preferably comprises at least four consecutive amino acids of Sequence ID. No. 3, and comprise at least five, six, seven or eight consecutive amino acids of Sequence ID. No. 3. Even more preferably, the fragment is between seven and twelve amino acids in length.

According to another aspect of the invention, methods for targeting an anti-*Pseudomonas* drug to a *Pseudomonas* microorganism is provided. The method involves contacting the environment of the *Pseudomonas* with a lipopolysaccharide-binding fragment as described above coupled to an anti-*pseudomonas* drug.

According to still another aspect of the invention, isolated nucleic acids are provided. The isolated nucleic acids encode the lipopolysaccharide-binding fragments of CFTR described above.

These and other aspects of the invention are described in greater detail below. It is noted that the isolated polysaccharides of the invention, in preferred embodiments, have the more detailed structures as described below in connection with the detailed description of the invention. These particular structures are considered important aspects of the invention.

### Detailed Description of the Invention

The invention involves the discovery that *P. aeruginosa* binds to CFTR and, more particularly, that the lipopolysaccharide (LPS) core of *P. aeruginosa* binds to CFTR. Surprisingly, it was discovered that contacting cells expressing the CFTR with the *P. aeruginosa* LPS core resulted in upregulation of the expression of CFTR. It also was discovered, surprisingly, that such upregulation results in improved uptake of *P. aeruginosa* by such cells and improved clearance of *P. aeruginosa* by such cells, thereby providing the basis of a therapy using the core of *P. aeruginosa* LPS to inhibit, prevent or otherwise treat infection by *P. aeruginosa*. Mammals pretreated with LPS core fragments are less susceptible to infection by *P. aeruginosa*. They also recover more quickly from infection by *P. aeruginosa* than animals without such pretreatment. As a result of the foregoing discoveries, methods and products are provided that make use of CFTR binding fragments of *P. aeruginosa* LPS cores. Methods and products also are provided that make use of LPS binding-fragments of cystic fibrosis transmembrane conductance regulators. (The mRNA and amino acid sequences of CFTR are provided in SEQ.ID.NO.1 and SEQ.ID.NO.2, respectively.)

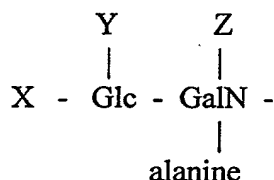
The methods and products of the invention are useful in connection with cells, microorganisms and subjects.

As used herein, a subject is a human, nonhuman primate, horse, cow, sheep, goat, dog, cat, or rodent.

As used herein in connection with polysaccharides and polypeptides, "isolated" means essentially free of other substances with which the polysaccharides or polypeptides may be found in nature or in *in vivo* systems to an extent practical and appropriate for their intended use. The material is sufficiently pure and sufficiently free of other biological materials so that it may be used in, for example, a pharmaceutical preparation. The material may be isolated using conventional techniques known to those of ordinary skill in the art. The material also may be prepared by synthetic chemistry using procedures known to those of ordinary skill in the art.

Because an isolated material may be admixed with a pharmaceutically acceptable carrier(s) in a pharmaceutical preparation, the material may comprise only a small percentage by weight of the preparation; it nevertheless still is isolated as is meant herein. An isolated fragment of a polypeptide or polysaccharide also is a portion of the polypeptide or polysaccharide as found in nature, isolated from the remaining portion as found in nature.

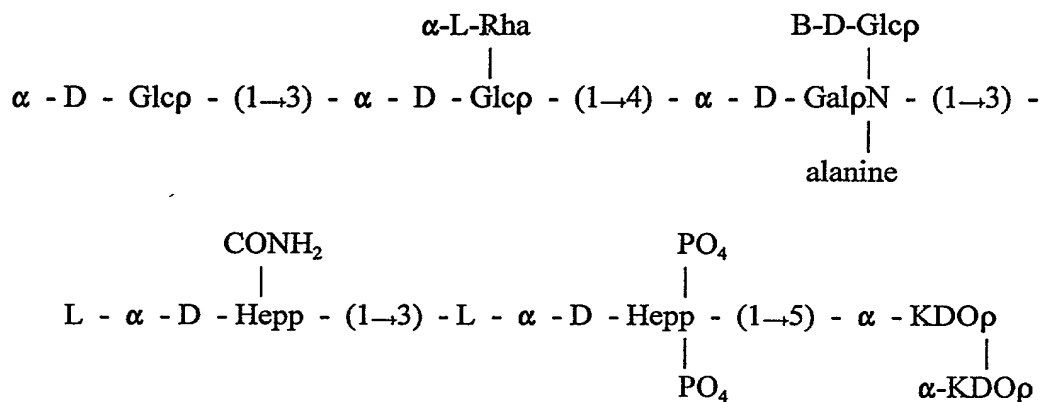
According to one aspect of the invention, a method for upregulating CFTR expression in a tissue of a subject is provided. The method involves administering to a subject in need of such upregulation a CFTR expression regulator in an amount effective to increase CFTR expression in the tissue. The CFTR expression regulator is an isolated polysaccharide that is an LPS core moiety comprising:



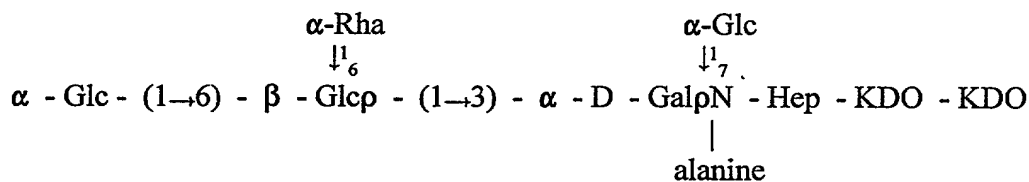
wherein X is selected from the group consisting of glucose, glucose-rhamnose and H; Y is selected from the group consisting of rhamnose and H; and Z is selected from the group consisting of glucose and H.

The entire core of the LPS of *P. aeruginosa* may be used, which consists essentially of the polysaccharide portion free of the lipid tail (which is somewhat toxic). For example, the isolated polysaccharide may be isolated from the O6 strain of *P. aeruginosa*, obtainable from the American Type Culture Collection, Rockville, Maryland (ATCC) under accession no. 33354. Mutant strains of *P. aeruginosa* also are available, which strains contain the essential portions of the polysaccharide of the invention as described above, such as, for example, *Pseudomonas* strain O3, ATCC accession no. 33350.

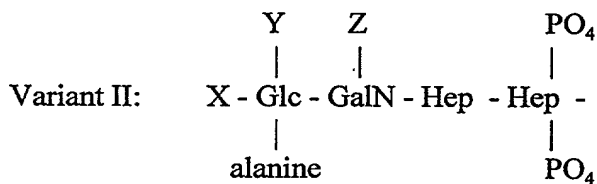
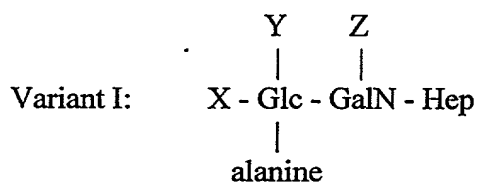
The structure of O6 is believed to be as follows:

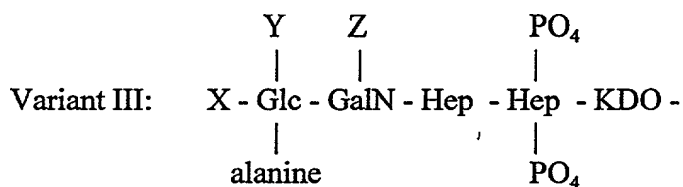


The structure of O3 is believed to be as follows:

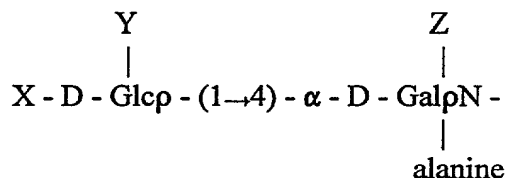


Variants derived from other mutant strains or prepared by chemical synthesis are useful according to the invention. The following variants are specific examples:





It is believed that the most preferable bonding configuration is



Those of ordinary skill in the art will be able to identify other variants and modifications useful according to the invention. Synthetic chemistry for constructing small polysaccharides is available. In addition, core polysaccharides can be derived from the many and various mutant strains of *P. aeruginosa*. These materials then may be simply tested for binding to the CFTR, the sequence of which is provided herein as Sequence ID. No. 1. The CFTR gene is the subject of gene therapy clinical protocols and has been studied extensively in various expression systems, many of which would be suitable for screening LPS core variant binding. The CFTR or the polysaccharide also could be bound to a substrate, such as a polystyrene plate. Screening experiments could involve direct measurement of the binding of the variant to CFTR if the variant were labeled, such as with a radioactive label or a florescent label. Likewise, the CFTR could be labeled in direct binding studies. Screening also can be carried out by measuring indirect binding such as in a competitive binding assay. Such assays could involve competition with *P. aeruginosa* binding to CFTR or with isolated core LPS of *P. aeruginosa* binding to CFTR. Those of ordinary skill in the art will readily know the details of such screening assays.

As mentioned above, the invention involves the surprising discovery that the polysaccharides of the invention upregulate CFTR expression and can result in increased uptake of *P. aeruginosa* and clearance of *P. aeruginosa*. Increased CFTR expression can be evaluated,

for example, by measuring CFTR mRNA, by using antibodies against the CFTR, or by measuring LPS core binding to the CFTR. Such measurements are well within the ability of those of ordinary skill in the art.

The invention is useful in treating a variety of conditions involving the CFTR. Most notably, the invention is useful whenever it is desirable to prevent, inhibit or halt infection by *P. aeruginosa*. This includes treating subjects having conditions that predispose the subjects to infection by *P. aeruginosa*, such as patients requiring medical intensive care (including surgical intensive care), patients with a basic compromise to the respiratory tract (such as by intubation), patients are immuno-compromised such as by anticancer chemotherapy treatment and patients with chronic obstructive pulmonary disease. Treatment can be prophylactic or can be concurrent with active infection. It is believed that frequent (daily) treatment will be most successful.

In the foregoing instances, the patients can have normal CFTRs. The invention is also useful when the patient's CFTRs are not normal, such as with cystic fibrosis patients who have a defective CFTR gene. In some instances, it is believed that a defective CFTR still will bind *P. aeruginosa*, although weakly and inadequately. Thus, upregulation of a defective CFTR also will enhance clearance of *P. aeruginosa*, and, therefore, the polysaccharides of the invention are useful in treating subjects with defective CFTR genes.

It is noted that CFTR is expressed in a variety of tissues and is believed to have functions in addition to simply binding and clearing *P. aeruginosa*. The receptor is expressed in the gastrointestinal tract (intestinal epithelial cells), in the goblets within the respiratory tract, in genital tissue, in kidney tissue, in pancreas tissue, in liver tissue, and in corneal epithelial cells. Some of the negative effects of a defective CFTR gene are managed by diet, and it is believed that upregulation of the defective receptor would be of benefit in these situations. Negative effects of a defective CFTR include malabsorption in the gastrointestinal tract (managed by diet and by patients taking pancreatic supplements, but patients are still usually in the lower 5th percentile for weight and height); sterility in males (genitourinary tract); on occasion, diabetes (pancreas) and arthritis. The polysaccharides of the invention, therefore, are useful whenever it is desirable to upregulate CFTR expression, whether the CFTR gene is normal or defective.

The presence of the CFTR in a variety of tissues also has led to another aspect of the invention, that is the use of the isolated polysaccharides of the invention to target a bioactive molecule to a tissue expressing a CFTR. The CFTR binding fragment of a LPS of *P. aeruginosa* is used as a targeting moiety in an otherwise conventional manner, to target bioactive agents to tissues expressing the CFTR. In general the targeting moiety is coupled to the bioactive agent. The molecules may be directly coupled to one another, such as by conjugation or may be indirectly coupled to one another where, for example, the targeting moiety is on the surface of the liposome and the bioactive agent is contained within the liposome. Thus, the invention contemplates conjugates of the isolated polysaccharides of the invention with bioactive agents. If the molecules are linked to one another, then the targeting moiety is covalently or noncovalently bound to the bioactive agent in a manner that preserves the targeting specificity of the targeting moiety. As used herein, "linked" or "linkage" means two entities are bound to one another by any physiochemical means. It is important that the linkage be of such a nature that it does not impair substantially the effectiveness of the bioactive molecule or the binding specificity of the targeting moiety. Keeping these parameters in mind, any linkage known to those of ordinary skill in the art may be employed, covalent or noncovalent. Such means and methods of linkage are well known to those of ordinary skill in the art.

Linkage according to the invention need not be direct linkage. The components of the compositions of the invention may be provided with functionalized groups to facilitate their linkage and/or linker groups may be interposed between the components of these compositions to facilitate their linkage. In addition, the components of the present invention may be synthesized in a single process, whereby the components could be regarded as one in the same entity. For example, a targeting moiety specific for a CFTR receptor could be synthesized together with the bioactive agent. These and other modifications are intended to be embraced by the present invention.

Specific examples of covalent bonds include those wherein bifunctional cross-linker molecules are used. The cross-linker molecules may be homobifunctional or heterobifunctional, depending upon the nature of the molecules to be conjugated. Homobifunctional cross-linkers have two identical reactive groups. Heterobifunctional cross-linkers have two different reactive

groups that allow sequential conjugation reaction. Various types of commercially available cross-linkers are reactive with one or more of the following groups: primary amines, secondary amines, sulfhydryls, carboxyls, carbonyls and carbohydrates.

Non-covalent methods of conjugation also may be used to join the targeting moiety and the bioactive agent. Non-covalent conjugation may be accomplished by direct or indirect means including hydrophobic interaction, ionic interaction, intercalation, binding to major or minor grooves of a nucleic acid and other affinity interactions.

Covalent linkages may be noncleavable in physiological environments or cleavable in physiological environments, such as linkers containing disulfide bonds. Such molecules may resist degradation and/or may be subject to different intracellular transport mechanisms. One of ordinary skill in the art will be able to ascertain without undue experimentation the preferred bond for linking the targeting moiety and the bioactive agent, based on the chemical properties of the molecules being linked and the preferred characteristics of the bond.

For indirect linkage, the targeting moiety may be part of a particle, such as a liposome, which targets the liposome to tissues expressing the CFTR. The liposome, in turn, may contain the bioactive agent. The manufacture of liposomes containing bioactive agents is fully described in the literature. Many are based upon cholesteric molecules as starting ingredients and/or phospholipids. They may be synthetically derived or isolated from natural membrane components. According to an embodiment of the invention, the polysaccharide which is the targeting moiety is attached to a non-toxic, lipophilic anchor that is biocompatible with a human subject. Virtually any hydrophobic substance can be used, including cholesteric molecules, phospholipids and fatty acids preferably of medium chain length (12C-20C). Preferred are naturally occurring fatty acids of between 14 and 18 carbons in length. These molecules can be attached to the polysaccharides of the invention, with the lipophilic anchor inserting into the membrane of a liposome and the polysaccharide tethered on the surface of the liposome for targeting the liposome to the cell expressing the CFTR.

It is noted that lipid A normally is attached to native lipopolysaccharide of *P. aeruginosa*. Lipid A, however, is somewhat toxic, and it is preferred that the polysaccharides of the invention, if attached to lipophilic molecules, be attached to molecules which have a better



toxicity profile than Lipid A. Useful lipophilic moieties may include any short to medium-chain length saturated fatty acid of the general composition  $\text{CH}_3(\text{CH}_2)_n\text{COOH}$  where n can range from 0-50. Preferably n will range from 1-20. In addition, the  $\text{CH}_2$  groups may be modified to be linked to each other through double-carbon bonds, making the fatty acid of the unsaturated category. Further modifications could include additional substituents such as hydroxyl (OH) groups added on to the fatty acid chain backbone to produce hydroxylated fatty acids. The substituents may be added to either the same side of the fatty acid chain (the trans-configuration) or to opposite sides of the fatty acid chain (the cis configuration). The lipophilic moieties may be attached to the targeting moieties as described above.

The bioactive agent may be virtually any bioactive agent useful in a cell expressing a CFTR. Bioactive agents, as used herein, include diagnostic agents such as radioactive labels and fluorescent labels. Bioactive agents also include molecules affecting the metabolism of a cell expressing a CFTR, including peptides, nucleic acids, and other natural and synthetic drug molecules. Included are: adrenergic agent; adrenocortical steroid; adrenocortical suppressant; alcohol deterrent; aldosterone antagonist; amino acid; ammonia detoxicant; anabolic; analeptic; analgesic; androgen; anesthesia, adjunct to; anesthetic; anorectic; antagonist; anterior pituitary suppressant; anthelmintic; anti-acne agent; anti-adrenergic; anti-allergic; anti-amebic; anti-androgen; anti-anemic; anti-anginal; anti-anxiety; anti-arthritis; anti-asthmatic; anti-atherosclerotic; antibacterial; anticholelithic; anticholelithogenic; anticholinergic; anticoagulant; anticoccidal; anticonvulsant; antidepressant; antidiabetic; antidiarrheal; antidiuretic; antidote; anti-emetic; anti-epileptic; anti-estrogen; antifibrinolytic; antifungal; antiglaucoma agent; antihemophilic; antihemorrhagic; antihistamine; antihyperlipidemia; antihyperlipoproteinemic; antihypertensive; antihypotensive; anti-infective; anti-infective, topical; anti-inflammatory; antikeratinizing agent; antimalarial; antimicrobial; antimigraine; antimitotic; antimycotic, antinauseant, antineoplastic, antineutropenic, antiobessional agent; antiparasitic; antiparkinsonian; antiperistaltic, antipneumocystic; antiproliferative; antiprostatic hypertrophy; antiprotozoal; antipruritic; antipsychotic; antirheumatic; antischistosomal; antiseborrheic; antisecretory; antispasmodic; antithrombotic; antitussive; anti-ulcerative; anti-urolithic; antiviral; appetite suppressant; benign prostatic hyperplasia therapy agent; blood

glucose regulator; bone resorption inhibitor; bronchodilator; carbonic anhydrase inhibitor; cardiac depressant; cardioprotectant; cardiotonic; cardiovascular agent; choleretic; cholinergic; cholinergic agonist; cholinesterase deactivator; coccidiostat; cognition adjuvant; cognition enhancer; depressant; diagnostic aid; diuretic; dopaminergic agent; ectoparasiticide; emetic; enzyme inhibitor; estrogen; fibrinolytic; fluorescent agent; free oxygen radical scavenger; gastrointestinal motility effector; glucocorticoid; gonad-stimulating principle; hair growth stimulant; hemostatic; histamine H2 receptor antagonists; hormone; hypocholesterolemic; hypoglycemic; hypolipidemic; hypotensive; imaging agent; immunizing agent; immunomodulator; immunoregulator; immunostimulant; immunosuppressant; impotence therapy adjunct; inhibitor; keratolytic; LHRH agonist; liver disorder treatment; luteolysin; memory adjuvant; mental performance enhancer; mood regulator; mucolytic; mucosal protective agent; mydriatic; nasal decongestant; neuromuscular blocking agent; neuroprotective; NMDA antagonist; non-hormonal sterol derivative; oxytocic; plasminogen activator; platelet activating factor antagonist; platelet aggregation inhibitor; post-stroke and post-head trauma treatment; potentiator; progestin; prostaglandin; prostate growth inhibitor; prothyrotropin; psychotropic; pulmonary surface; radioactive agent; regulator; relaxant; repartitioning agent; scabicide; sclerosing agent; sedative; sedative-hypnotic; selective adenosine A1 antagonist; serotonin antagonist; serotonin inhibitor; serotonin receptor antagonist; steroid; stimulant; suppressant; symptomatic multiple sclerosis; synergist; thyroid hormone; thyroid inhibitor; thyromimetic; tranquilizer; amyotrophic lateral sclerosis agent; cerebral ischemia agent; Paget's disease agent; unstable angina agent; uricosuric; vasoconstrictor; vasodilator; vulnerary; wound healing agent; xanthine oxidase inhibitor.

In one preferred embodiment, a gene under the control of a promoter, preferably in a plasmid, is coupled to the targeting moiety for delivering the gene to the cell expressing the CFTR. In one particularly important embodiment, the gene is a normal CFTR gene and the methods and products of the invention are used to treat subjects with defective CFTR genes by gene therapy. A gene therapy construct for CF, for example, may include either the cDNA sequence of CFTR incorporated into an appropriate expression system, or the genomic DNA sequence of CFTR including the coding exons and noncoding introns incorporated into an

appropriate expression vector. It could also be a construct containing only a portion of the CFTR that is needed to restore normal cellular function. For example, the first 150 amino acids are not needed for chloride ion conductance of the cell and this portion of CFTR could be produced from an appropriate cDNA or genomic DNA. Alternately, the portion of CFTR encoding the *P. aeruginosa* binding site (amino acids 103-118) could be expressed only in lung cells to promote resistance to infection since the rest of the molecule, which has ion-secretion properties, is not needed for resistance to infection. Antisense molecules can be delivered according to the methods of the invention as well. Thus, an important aspect of the invention is the targeting and delivery of oligonucleotides to cells expressing the CFTR.

Because of the discovery that CFTR binds *P. aeruginosa* LPS core, this has led to the further aspects of the invention related to the use of CFTR or CFTR fragments for therapeutic, diagnostic and research purposes as well as *in vivo* and *in vitro* methods relating thereto. Thus, according to another aspect of the invention, compositions of matters are provided that involve CFTR and fragments of the CFTR. In one aspect of the invention, a covalent conjugate of an anti-*Pseudomonas* drug and CFTR or a *Pseudomonas* lipopolysaccharide-binding fragment of CFTR is provided. The lipopolysaccharide-binding fragment comprises at least four consecutive amino acids of SEQ ID NO. 3. The fragment can comprise at least five consecutive amino acids, at least six consecutive amino acids, at least seven consecutive amino acids, or at least eight consecutive amino acids of SEQ ID NO. 3. SEQ ID NO. 3 consists of amino acids numbered 103-117 of the coding region of the CFTR. Thus, where at least four consecutive amino acids from SEQ ID NO. 3 are involved, there are twelve possibilities as follows: amino acids numbered 103-106, 104-107, 105-108, 106-109, 107-110, 108-111, 109-112, 110-113, 111-114, 112-115, 113-116 and 114-117. Where at least five consecutive amino acids are involved, there are eleven possibilities as follows: amino acids numbered 103-107, 104-108, 105-109, 106-110, 107-111, 108-112, 109-113, 110-114, 111-115, 112-116 and 113-117. Where there are at least six consecutive amino acids involved, there are ten possibilities as follows: amino acids numbered 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116 and 112-117. Where there are at least seven consecutive amino acids involved, there are nine possibilities as follows: amino acids numbered 103-109, 104-110, 105-111, 106-112, 107-113,

108-114, 109-115, 110-116 and 111-117. Where there are eight consecutive amino acids involved, there are eight possibilities as follows: amino acids numbered 103-110, 104-111, 105-112, 106-113, 107-114, 108-115, 109-116 and 110-117.

It is believed that the optimal fragments will be between six and twelve amino acids in length, preferably between six and eight amino acids in length.

Determining the optimum sequence and number of amino acids for optimum binding to LPS can be determined with no more than routine experimentation. Segments of SEQ ID NO. 3 can be readily synthesized and binding experiments with, for example, immobilized *P. aeruginosa* lipopolysaccharide core can be carried out. The assay can be direct, as with radioactive labeled fragments of SEQ ID NO. 3 or can be indirect such as using competitive binding assays between SEQ ID NO. 3 and fragments of SEQ ID NO. 3. The invention thus contemplates the essential and optimal binding fragment of SEQ ID NO. 3.

The isolated lipopolysaccharide-binding fragments of a cystic fibrosis transmembrane conductance regulator can be labeled or coupled to a drug for targeting *P. aeruginosa* *in vitro* or *in vivo*. The anti-*Pseudomonas* drug can be any drug effective in either diagnosing a *Pseudomonas* infection or in treating a *Pseudomonas* infection. Such drugs include antimicrobials, antimicrobial potentiating agents, immune system recognition enhancers, diagnostic molecules and the like.

Molecules useful as antimicrobials can be delivered by the methods and compositions of the invention, such that the pathogenic infection is reduced or eliminated. Anti-*Pseudomonas* agent drugs can be bactericidal or bacteriostatic by inhibiting replication of bacteria or inhibiting synthesis of bacterial components required for survival of the infecting organism.

Anti-*Pseudomonas* antibiotic include the following: Amifloxacin; Amifloxacin Mesylate; Amikacin; Amikacin Sulfate; Aminosalicic acid; Aminosalicylate sodium; Azlocillin; Azlocillin Sodium; Bacampicillin Hydrochloride; Carbenicillin Disodium; Carbenicillin Indanyl Sodium; Carbenicillin Phenyl Sodium; Carbenicillin Potassium; Carumonam Sodium; Cefaclor; Cefadroxil; Cefamandole; Cefamandole Nafate; Cefamandole Sodium; Cefaparolet; Cefatrizine; Cefazaflur Sodium; Cefazolin; Cefazolin Sodium; Cefbuperazone; Cefdinir; Cefepime; Cefepime Hydrochloride; Cefetecol; Cefixime; Cefmenoxime Hydrochloride; Cefmetazole; Cefmetazole

Sodium; Cefonicid Monosodium; Cefonicid Sodium; Cefoperazone Sodium; Ceforanide; Cefotaxime Sodium; Cefotetan; Cefotetan Disodium; Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceftizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalexin; Cephalexin Hydrochloride; Cephaloglycin; Cephaloridine; Cephalothin Sodium; Cephapirin Sodium; Cephradine; Cetocycline Hydrochloride; Cetophenicol; Chlortetracycline Bisulfate ; Chlortetracycline Hydrochloride ; Ciprofloxacin; Ciprofloxacin Hydrochloride; Colistin Sulfate; Coumermycin; Coumermycin Sodium; Doxycycline; Doxycycline Calcium; Doxycycline Fosfatex; Doxycycline Hyclate; Droxacin Sodium; Enoxacin; Epicillin; Epitetracycline Hydrochloride; Imipenem; Kanamycin Sulfate; Meclocycline; Minocycline; Minocycline Hydrochloride; Nafcillin Sodium; Norfloxacin; Ofloxacin; Oxytetracycline; Oxytetracycline Calcium; Piperacillin Sodium; Pirbenicillin Sodium; Tetracycline; Tetracycline Hydrochloride ; Tetracycline Phosphate Complex; Ticarcillin Cresyl Sodium; Ticarcillin Disodium; Ticarcillin Monosodium; Tobramycin; and Tobramycin Sulfate.

The foregoing molecules may be coupled to CFTR or the fragment of CFTR that acts as the targeting moiety by the methods as described above in connection with the LPS-core targeting moiety.

The invention also involves isolated nucleic acids that encode the fragments of CFTR described above. Such nucleic acids are useful in producing the fragments described above. The nucleic acids can be part of an expression vector and can be included within a host cell. Virtually any cells, prokaryotic or eukaryotic, which can be transformed with CFTR DNA or RNA and which can be grown or maintained in culture may be used in the practice of the invention. Examples include bacterial cells such as E. coli and mammalian cells such as mouse, hamster, pig, goat, primate, etc. Specific examples include CHO cells and COS cells. Cell-free transcription systems also may be used in lieu of cells.

When used therapeutically, the compounds of the invention are administered in therapeutically effective amounts. In general, a therapeutically effective amount means that

amount necessary to delay the onset of, inhibit the progression of, ameliorate the symptoms of or halt altogether the particular condition being treated. It is less than that amount that produces medically unacceptable side-effects. Generally, a therapeutically effective amount will vary with the subject's age, condition, and sex, as well as the nature and extent of the disease in the subject, all of which can be determined by one of ordinary skill in the art. The dosage may be adjusted by the individual physician or veterinarian, particularly in the event of any complication. A therapeutically effective amount typically varies from 0.01 mg/kg to about 1000 mg/kg, preferably from about 0.1 mg/kg to about 200 mg/kg and most preferably from about 0.2 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or more days.

The therapeutics of the invention can be administered by any conventional route, including injection or by inhalation. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When the subject has cystic fibrosis, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the therapeutic, such as the binding capacity of the polysaccharide (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing aerosols without resort to undue experimentation.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The invention also contemplates gene therapy. The procedure for performing ex vivo gene therapy is outlined in U.S. Patent 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction in vitro of a functional copy of a gene into a cell(s) of a subject which contains a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. In vivo gene therapy also is contemplated according to the invention. Gene therapy for cystic fibrosis is underway in clinical trials, and various vectors for expressing CFTR are known to those skilled in the art.

Certain of the various objects and advantages of the invention are illustrated in the following examples. Numerous equivalents and embodiments will be apparent to those of ordinary skill in the art and are intended to be embraced by the appended claims.

### **Examples**

**Example 1:** CFTR is the cellular receptor for *P. aeruginosa* internalization.

#### ***Materials and methods:***

**Cell lines.** CFT1-LCFSN cells, carrying a retrovirally introduced chromosomal copy of the wild-type human CFTR gene were kindly provided by J. Olsen, J. Yankaskis and L. Johnson from The University of North Carolina, Chapel Hill, NC [8,9]. The cell line parental to CFT1-LCFSN is designated CFT1; it is a line of human papilloma virus 18 E6/E7 transformed bronchial epithelial cells derived from a CF patient homozygous for  $\Delta F508$  CFTR. The CFT1-LCFSN cells have normal chloride ion conductance [8, 9]. CFT1- $\Delta F508$  cells are derived from CFT1 cells and carry a cDNA introduced by a retrovirus vector that encodes the  $\Delta F508$  mutant form of CFTR. Cells were grown in supplemented F-12 medium as described [8] in 5% CO<sub>2</sub> at 37°C.

C127 cells expressing wild-type or  $\Delta F508$  CFTR were obtained from Genzyme Corp., Framingham, MA. [10] C127 cells were grown in RPMI medium with 2.5 grams dextrose/liter, supplemented with glutamine, non-essential amino acids, sodium pyruvate, 2-mercaptoethanol, 10% fetal bovine serum and 400  $\mu$ g G418/ml. Parental cells were grown without the G418. Cells were released from monolayers in tissue culture flasks by 5 min. of incubation with trypsin- versene mixture (BioWhitaker, Walkersville, MD), washed, counted and seeded into 96-well tissue culture plates at  $10^5$  cells/well in supplemented F-12 medium and incubated at 37°C in 5% CO<sub>2</sub>.

WI-38 (human diploid lung cell line, ATCC no. CCL-75) and A549 cells (human lung carcinoma cells, ATCC no. CCL-185) were obtained from the ATCC and grown according to their instructions.

**Bacterial strains.** *P. aeruginosa* strains used include PAO1, a well-characterized laboratory strain, and strains 149 and 324, non-mucoid, LPS-smooth clinical isolates of *P. aeruginosa* from CF patients early in the course of infection. Fresh cultures of *P. aeruginosa* grown overnight at 37°C on a tryptic soy agar plate were suspended in supplemented medium to prepare the bacterial inoculum. Approximately  $10^6$  cfu of the bacterial inoculum were added per well of  $10^5$  epithelial cells.

**Reagents for inhibition of ingestion assays.** Membranes were prepared from C127 cells as described by O'Riordan et al. [11]. Membranes were suspended in 150 mM NaCl, 50 mM Tris, pH 7.5 and 1 mM EDTA and added at the indicated concentration to suspensions of *P. aeruginosa* strains prior to adding this mixture to CFT1-LCFSN cells to measure bacterial uptake. Membranes from CEM/Vbl cells expressing P-glycoprotein and membranes from control CEM cells lacking P-glycoprotein were obtained from James Croop of Harvard Medical School. Purified (~85%) recombinant CFTR was obtained from Genzyme Corp., and prepared as described [11]. The protein was solubilized in 100 mM NaCl, 10 mM Tris, pH 8.0, 2 mM di-thio-threitol and 0.1 % sodium dodecyl sulfate. Monoclonal antibodies raised to synthetic peptides corresponding to the first (MAb CF3) and fourth (MAb CF4) predicted extracellular domains of CFTR, as well as a MAb specific to a peptide representing the carboxy-terminal 14 amino acids of mature CFTR (MAb CF2) were provided by Dr. George Banting, University of



Bristol, Bristol, UK [12]. Synthetic peptides were obtained from Chiron Mimetopes, San Diego, CA. Peptide GRIASYDPDNKEER (15 amino acids) (SEQ.ID.NO. 3) represents amino acids 103-117 of mature CFTR; peptide LWLLGNTPLQDKGNSTHSRNNSYAVIITSTS (31 amino acids) (SEQ.ID.NO. 4) represents amino acids 881-911 of mature CFTR. Peptides were made up as a stock solution in F-12 medium containing 1  $\mu\text{M}/\mu\text{l}$  based on the purity reported by the manufacturer. Peptides were diluted in F-12 tissue culture medium prior to use in assays.

**Bacterial ingestion assay.** Cells were released from monolayers in tissue culture flasks by 5 min. of incubation with trypsin-versene mixture (BioWhitaker, Walkersville, MD), washed, counted and seeded into 96-well tissue culture plates at  $10^5$  cells/well in supplemented F-12 medium [8] and incubated at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$ . Fresh cultures of *P. aeruginosa* grown overnight at  $37^\circ\text{C}$  on a tryptic soy agar plate were suspended in supplemented F-12 medium to prepare the bacterial inoculum. Then  $\sim 10^6$  colony forming units (cfu) of the bacterial inoculum were added per well of  $10^5$  epithelial cells. Bacteria were allowed to invade the epithelial cells for 3 to 4 hours at  $37^\circ\text{C}$ , after which nonadherent bacteria were removed by washing. The remaining steps of the assay and the steps involved in the controls have previously been described [13]. Three to 9 replicates were obtained per point, and analyzed using analysis of variance (ANOVA) and the Fisher PLSD statistic to determine pairwise differences [14].

Inhibition of ingestion in the presence of membranes isolated from C127 cells, monoclonal antibodies to extracellular domains of CFTR, or synthetic peptides corresponding to the first or fourth extracellular domains of CFTR was evaluated by adding these materials to the bacteria prior to adding them directly into wells for evaluation of bacterial uptake.

Augmentation of ingestion was tested by incubating cultures of  $10^5$  cells in 96-well plates with complete or incomplete lipopolysaccharide (LPS) core oligosaccharide isolated from *P. aeruginosa* strains PAC557 or PAC1R(*algC::tet*), [15] respectively, as described [7]. The oligosaccharides were added at various concentrations for 24 hours then cells washed extensively with tissue-culture medium prior to adding bacteria for internalization assays.

**Neonatal mouse model of infection.** Seven-day old neonatal Balb/c mice were infected with  $\sim 10^8$  cfu of strain PAO1 delivered intranasally as described [16], with the addition of 10 nM of a synthetic peptide corresponding to either the first or fourth extracellular domains of CFTR to

the bacterial inoculum. Twenty-four hours later 7 animals were killed, right and left lungs removed, weighed and dispersed into single cell suspensions by grinding through a fine-mesh wire screen. An aliquot was removed, Triton X-100 added to a final concentration of 0.5% to release intracellular bacteria and the total cfu of bacteria present in each lung determined. The remaining portion of the lung cell suspension was treated with 300 µg gentamicin/ml for 60 minutes to kill extracellular *P. aeruginosa*. The cells were then pelleted in a centrifuge (400 x g, 10 minutes), washed twice in RPMI medium, and resuspended in 200 µl of 0.5% Triton X-100 to release intracellular bacteria that survived the gentamicin treatment. These suspensions were diluted and plated for bacterial enumeration. The cfu per milligram lung weight was calculated and differences among groups analyzed by nonparametric statistics (Mann-Whitney U test) due to outliers in some groups [14].

### Results:

To determine if CFTR is a receptor for *P. aeruginosa* internalization, bacterial uptake assays using transformed murine epithelial C127 cells stably transfected with cDNA encoding either wild-type or ΔF508-mutant CFTR were carried out. Cells were prepared and treated as described above under the heading Bacterial Ingestion Assay and the amount of bacteria ingested by the cells was measured as mean colony forming units (CFU). In comparison to the parental C127 line (C127 parent), and the line transfected with mutant ΔF508 CFTR (C127-ΔF508), cells expressing wild-type human CFTR (C127-WT) had significantly enhanced uptake of three isolates of *P. aeruginosa* (Table 1). The data of Table 1 indicates that the WT CFTR is involved in the ingestion of *P. aeruginosa* because the cells transfected with the WT CFTR are capable of ingesting more *P. aeruginosa* than cells transfected with the mutant CFTR.

Table 1

Ingestion of various strains of <i>Pseudomonas aeruginosa</i> (Mean colony forming units (standard deviation) of <i>P. aeruginosa</i> strain ingested by the C127 cells)			
<i>P. aeruginosa</i>	C127 parent	C127-ΔF508	C127-WT
149	6.6X10 <sup>3</sup> (1.1355X10 <sup>3</sup> )	8.583X10 <sup>3</sup> (2.938X10 <sup>3</sup> )	7.2183X10 <sup>4</sup> (7.605X10 <sup>3</sup> )
324	7.517X10 <sup>3</sup> (3.339X10 <sup>3</sup> )	1.8767X10 <sup>4</sup> (2.655X10 <sup>3</sup> )	5.5717X10 <sup>4</sup> (1.4534X10 <sup>4</sup> )
PAO1	1.9X10 <sup>4</sup> (1.192X10 <sup>4</sup> )	4.6X10 <sup>4</sup> (1.59X10 <sup>4</sup> )	1X10 <sup>5</sup> (5.59X10 <sup>3</sup> )

Based on the above experiment, it was hypothesized that WT CFTR expressed on the cell surface might be interacting with the LPS. If this were correct, then exogenously added CFTR should be able to inhibit the interaction between *P. aeruginosa* and cells expressing the WT CFTR. To test this hypothesis membranes isolated from the three C127 cell lines were added to cultures of *P. aeruginosa* and then the mixture was added to the transformed human airway epithelial cell line CFT1-LCFSN (originally derived from a CF patient homozygous for the ΔF508 mutation and subsequently transfected with wild-type CFTR DNA) (Table 2). While incubating the *P. aeruginosa* with the membranes derived from C127 cells expressing wild-type CFTR inhibited epithelial cell uptake of *P. aeruginosa*, neither the C127 parent cells nor the C127-ΔF508 cells inhibited uptake. This suggests that the CFTR on the surface of airway epithelial cells is specifically interacting with the LPS and mediating its uptake.

Table 2

Inhibition of internalization of <i>P. aeruginosa</i> strain PAO1 into transformed human airway epithelial cells (CFT1-LCFSN line). [Mean cfu <i>P. aeruginosa</i> internalized (standard deviation			
Amount Inhibitor	Parental cells (no CFTR)	$\Delta$ F508 CFTR	Wild-type CFTR
250 $\mu$ g	$7.49 \times 10^4$ ( $1.2878 \times 10^4$ )	$9.3383 \times 10^4$ ( $2.0009 \times 10^4$ )	$1.0383 \times 10^4$ ( $1.986 \times 10^3$ ) <sup>a</sup>
100 $\mu$ g	$8.0117 \times 10^4$ ( $1.3263 \times 10^4$ )	$6.7533 \times 10^4$ ( $6.426 \times 10^3$ )	$4.6683 \times 10^4$ ( $6.53 \times 10^3$ ) <sup>a</sup>
25 $\mu$ g	$8.375 \times 10^4$ ( $3.5746 \times 10^4$ )	$8.1133 \times 10^4$ ( $2.2992 \times 10^4$ )	$5.6983 \times 10^4$ ( $2.409 \times 10^3$ ) <sup>a</sup>
10 $\mu$ g	$6.535 \times 10^4$ ( $3.481 \times 10^3$ )	$6.39 \times 10^4$ ( $5.92 \times 10^3$ )	$6.595 \times 10^4$ ( $5.914 \times 10^3$ )

## Controls

No inhibitor	$9.858 \times 10^4$ ( $2.228 \times 10^4$ )
P glycoprotein <sup>b</sup>	$9.2567 \times 10^4$ ( $3.5815 \times 10^4$ )
Control membranes <sup>c</sup>	$9.3217 \times 10^4$ ( $1.9863 \times 10^4$ )

<sup>a</sup>, Significantly less cfu of *P. aeruginosa* internalized compared to inhibition with membranes from parental C127 cells or membranes from C127 cells expressing the  $\Delta$ F508 mutant of CFTR at  $P < .01$ , ANOVA

<sup>b</sup>, P glycoprotein in 50  $\mu$ g of membranes from CEM/Vbl cells

<sup>c</sup>, control membranes from CEM cells lacking P-glycoprotein

Furthermore, when highly purified (~85%) recombinant CFTR was added to a culture of *P. aeruginosa* prior to addition to tissue culture wells containing CFT1-LCFSN cells, significant inhibition of bacterial ingestion was obtained with nanogram quantities of CFTR (Table 3). These results strongly implicate CFTR as the epithelial-cell ligand for internalization of *P. aeruginosa*.

Table 3

Inhibition of internalization of <i>P. aeruginosa</i> strain PAO1 into transformed human airway epithelial cells (CFT1-LCFSN line) by highly purified recombinant CFTR mean cfu <i>P. aeruginosa</i> internalized (standard deviation)		
CONCENTRATION OF PROTEIN / ml	BSA	CFTR
5000 µg protein	9.2 X 10 <sup>4</sup> (3.9 X 10 <sup>4</sup> )	1.65 X 10 <sup>4</sup> (1.05 X 10 <sup>4</sup> ) <sup>a</sup>
625 µg protein	8.3 X 10 <sup>4</sup> (1.65 X 10 <sup>4</sup> )	4.9 X 10 <sup>4</sup> (1.25 X 10 <sup>4</sup> ) <sup>a</sup>
78 µg protein	9.55 X 10 <sup>4</sup> (2.55 X 10 <sup>4</sup> )	8.55 X 10 <sup>4</sup> (1.15 X 10 <sup>4</sup> )
9.7 µg protein	8.55 X 10 <sup>4</sup> (2.8 X 10 <sup>4</sup> )	1.05 X 10 <sup>5</sup> (2.5 X 10 <sup>4</sup> )
(control) 0.0 (Tissue culture media only)	1.58 X 10 <sup>5</sup> (3.6 X 10 <sup>4</sup> )	
0.0 (Media + protein solubilization buffer) <sup>b</sup>	1.475 X 10 <sup>5</sup> (1.4 X 10 <sup>4</sup> )	

<sup>a</sup>, Significantly different from inhibition with BSA, P < .01, unpaired t-test.

<sup>b</sup>, Tissue culture media plus 0.1% Tris-SDS buffer used to solubilize CFTR

#### Example 2: Identification of the domain of CFTR that interacts with *P. aeruginosa*

##### Results:

To identify the extracellular domain of CFTR that interacts with *P. aeruginosa*, monoclonal antibodies (Mab) were raised to synthetic peptides corresponding to the first (Mab CF3) and fourth (Mab CF4) predicted extracellular domains of CFTR, as well as a Mab specific to a peptide representing the carboxy-terminal 14 amino acids of mature CFTR (Mab CF2) [12]. Addition of these MAbs in various concentrations to cultures of three *P. aeruginosa* strains prior to their addition to the CFT1-LCFSN cells resulted in a concentration-dependent inhibition of internalization of *P. aeruginosa* by Mab CF3. This inhibitory effect was not observed with the other MAbs (Tables 4A, 4B and 4C).

Table 4A

<b>Inhibition of internalization of <i>P. aeruginosa</i> strain PAO1 into transformed human airway epithelial cells (CFT1-LCFSN line) by monoclonal antibodies (MAbs) specific to extracellular domains of CFTR</b> <b>[mean cfu of <i>P. aeruginosa</i> internalized (standard deviation)]</b>			
MAb dilution	C-terminus (Mab CF2)	4th Outer Domain (Mab CF4)	First Outer Domain (Mab CF3)
2	4.51 X 10 <sup>4</sup> (8.061 X 10 <sup>3</sup> )	4.75 X 10 <sup>4</sup> (5.491 X 10 <sup>3</sup> )	4.45 X 10 <sup>3</sup> (1.307 X 10 <sup>3</sup> ) <sup>a</sup>
10	4.4517X10 <sup>4</sup> (5.283X10 <sup>3</sup> )	5.085 X 10 <sup>4</sup> (3.369 X 10 <sup>3</sup> )	1.4667 X 10 <sup>4</sup> (4.556 X 10 <sup>3</sup> ) <sup>a</sup>
25	5.0733 X 10 <sup>4</sup> (3.721 X 10 <sup>3</sup> )	5.145 X 10 <sup>4</sup> (4.06 X 10 <sup>3</sup> )	4.285 X 10 <sup>4</sup> (3.635 X 10 <sup>3</sup> ) <sup>a</sup>
100	4.6067 X 10 <sup>4</sup> (6.397 X 10 <sup>3</sup> )	4.7583 X 10 <sup>4</sup> (6.651 X 10 <sup>3</sup> )	4.908 X 10 <sup>4</sup> (3.832 X 10 <sup>3</sup> )
0	5.6933 X 10 <sup>4</sup> (4.505 X 10 <sup>3</sup> )	5.6933 X 10 <sup>4</sup> (4.505 X 10 <sup>3</sup> )	5.6933 X 10 <sup>4</sup> (4.505 X 10 <sup>3</sup> )

<sup>a</sup>, Significantly fewer internalized *P. aeruginosa* bacteria in the presence of MAbs specific to the other domains of CFTR, or no MAb at P < .001, ANOVA

**Table 4B**

<b>Inhibition of internalization of <i>P. aeruginosa</i> strain 149 into transformed human airway epithelial cells (CFT1-LCFSN line) by monoclonal antibodies (MAbs) specific to extracellular domains of CFTR</b> <b>[mean cfu of <i>P. aeruginosa</i> internalized (standard deviation)]</b>			
MAb dilution	C-terminus (Mab CF2)	4th Outer Domain (Mab CF4)	First Outer Domain (Mab CF3)
2	1.1067 X 10 <sup>4</sup> (1.61 X 10 <sup>3</sup> )	1.8033 X 10 <sup>4</sup> (1.041 X 10 <sup>3</sup> )	6.7 X 10 <sup>1</sup> (1.21 X 10 <sup>2</sup> ) <sup>a</sup>
10	1.6117 X 10 <sup>4</sup> (2.568 X 10 <sup>3</sup> )	1.6517 X 10 <sup>4</sup> (2.372 X 10 <sup>3</sup> )	3.33 X 10 <sup>2</sup> (3.78 X 10 <sup>2</sup> ) <sup>a</sup>
25	1.375 X 10 <sup>4</sup> (1.78 X 10 <sup>3</sup> )	1.4586 X 10 <sup>4</sup> (5.989 X 10 <sup>3</sup> )	6.067 X 10 <sup>3</sup> (5.85 X 10 <sup>2</sup> ) <sup>a</sup>
100	1.57 X 10 <sup>4</sup> (2.338 X 10 <sup>3</sup> )	1.675 X 10 <sup>4</sup> (1.924 X 10 <sup>3</sup> )	1.6233 X 10 <sup>4</sup> (1.532 X 10 <sup>3</sup> )
0	1.685 X 10 <sup>4</sup> (2.512 X 10 <sup>3</sup> )	1.685 X 10 <sup>4</sup> (2.512 X 10 <sup>3</sup> )	1.685 X 10 <sup>4</sup> (2.512 X 10 <sup>3</sup> )

<sup>a</sup>, Significantly fewer internalized *P. aeruginosa* bacteria in the presence of MAbs specific to the other domains of CFTR, or no MAb at P < .001, ANOVA

Table 4C

<b>Inhibition of internalization of <i>P. aeruginosa</i> strain 324 into transformed human airway epithelial cells (CFT1-LCFSN line) by monoclonal antibodies (MAbs) specific to extracellular domains of CFTR</b> <b>[mean cfu of <i>P. aeruginosa</i> internalized (standard deviation)]</b>			
MAb dilution	C-terminus (MAb CF2)	4th Outer Domain (MAb CF4)	First Outer Domain (MAb CF3)
2	$3.0317 \times 10^4 (3.075 \times 10^3)$	$3.6083 \times 10^4 (3.226 \times 10^3)$	$6.933 \times 10^3 (9.91 \times 10^2)^a$
10	$3.7317 \times 10^4 (3.471 \times 10^3)$	$4.97 \times 10^4 (2.695 \times 10^3)$	$1.575 \times 10^4 (1.5 \times 10^3)^a$
25	$3.4433 \times 10^4 (3.335 \times 10^3)$	$3.5633 \times 10^4 (2.553 \times 10^3)$	$3.0767 \times 10^4 (2.934 \times 10^3)$
100	$3.9167 \times 10^4 (2.962 \times 10^3)$	$3.7067 \times 10^4 (3.964 \times 10^3)$	$3.36 \times 10^4 (4.177 \times 10^3)$
0	$3.4867 \times 10^4 (2.422 \times 10^3)$	$3.4867 \times 10^4 (2.422 \times 10^3)$	$3.4867 \times 10^4 (2.422 \times 10^3)$

<sup>a</sup>, Significantly fewer internalized *P. aeruginosa* bacteria in the presence of MAbs specific to the other domains of CFTR, or no MAb at  $P < .001$ , ANOVA

To confirm the identification of this domain of CFTR as the binding site for *P. aeruginosa*, peptides corresponding to the first and fourth extracellular domains were synthesized for use in internalization-inhibition assays. Picomole quantities of the synthetic peptide corresponding to the first, but not the fourth, predicted extracellular domain of CFTR inhibited epithelial cell internalization of *P. aeruginosa* (Table 5), suggesting that the binding site resides in the first extracellular domain.



Table 5

<b>Inhibition of internalization of <i>P. aeruginosa</i> strain PAO1 into transformed human airway epithelial cells (CFT1-LCFSN line) by synthetic peptides corresponding to the first or fourth predicted extracellular domains of CFTR</b> (Mean cfu of <i>P. aeruginosa</i> internalized (standard deviation) in the presence of the synthetic peptide corresponding to the indicated extracellular domain of CFTR)		
Concentration of inhibitor (nanomoles)		
Strain PAO1	First domain	Fourth domain
No inhibitor	8.17 X 10 <sup>4</sup> (2.0861 X 10 <sup>3</sup> )	
1	2.38333 X 10 <sup>4</sup> (4.6207 X 10 <sup>3</sup> ) <sup>a</sup>	8.70833 X 10 <sup>4</sup> (4.0519 X 10 <sup>3</sup> )
0.1	2.51333 X 10 <sup>4</sup> (1.6609 X 10 <sup>3</sup> ) <sup>a</sup>	7.48833 X 10 <sup>4</sup> (5.0653 X 10 <sup>3</sup> )
0.01	3.48167 X 10 <sup>4</sup> (3.8473 X 10 <sup>3</sup> ) <sup>a</sup>	7.23 X 10 <sup>4</sup> (3.6721 X 10 <sup>3</sup> )
0.001	5.53333 X 10 <sup>4</sup> (5.2986 X 10 <sup>3</sup> )	6.97833 X 10 <sup>4</sup> (5.4745 X 10 <sup>3</sup> )
Strain 149	First domain	Fourth domain
No inhibitor	5.22833 X 10 <sup>4</sup> (5.6651 X 10 <sup>3</sup> )	
1	3.7833 X 10 <sup>3</sup> (4.491 X 10 <sup>2</sup> ) <sup>a</sup>	5.735 X 10 <sup>4</sup> (4.0009 X 10 <sup>3</sup> )
0.1	7.0167 X 10 <sup>3</sup> (8.542 X 10 <sup>2</sup> ) <sup>a</sup>	5.52833 X 10 <sup>4</sup> (6.0598 X 10 <sup>3</sup> )
0.01	1.705 X 10 <sup>4</sup> (2.1333 X 10 <sup>3</sup> ) <sup>a</sup>	4.86 X 10 <sup>4</sup> (4.6463 X 10 <sup>3</sup> )
0.001	5.73 X 10 <sup>4</sup> (4.006 X 10 <sup>3</sup> )	4.75167 X 10 <sup>4</sup> (5.9915 X 10 <sup>3</sup> )
Strain 324	First domain	Fourth domain
No inhibitor	1.467167 X 10 <sup>5</sup> (9.4252 X 10 <sup>3</sup> )	
1	1.46167 X 10 <sup>4</sup> (2.6955 X 10 <sup>3</sup> ) <sup>a</sup>	1.42 X 10 <sup>5</sup> (1.74624 X 10 <sup>4</sup> )
0.1	7.23167 X 10 <sup>4</sup> (5.6982 X 10 <sup>3</sup> ) <sup>a</sup>	1.416 X 10 <sup>5</sup> (1.19465 X 10 <sup>4</sup> )
0.01	1.405 X 10 <sup>5</sup> (9.4323 X 10 <sup>3</sup> )	1.412 X 10 <sup>5</sup> (7.1986 X 10 <sup>3</sup> )
0.001	1.3938 X 10 <sup>5</sup> (7.6735 X 10 <sup>3</sup> )	1.3275 X 10 <sup>5</sup> (9.408 X 10 <sup>3</sup> )

<sup>a</sup>, Significantly fewer internalized *P. aeruginosa* bacteria compared with bacteria inhibited by the fourth extracellular domain peptide at P < .01, ANOVA.

In order to verify the above results both the MAb and peptide experiments were repeated using two additional cell lines homozygous for wild-type CFTR, WI-38 diploid human embryonic lung cells and A549 human lung carcinoma cells. The data revealed an identical pattern to that observed using the CFT1-LCFSN cells (Table 6). It is of interest that the amino terminus of CFTR up to amino acid 150, including the first predicted extracellular domain, can be deleted from the molecule without affecting its ability to function as a chloride ion channel [17]. Thus CFTR-mediated cellular internalization of *P. aeruginosa* is unrelated to the well-described ion-channel properties of this molecule.

**Table 6**

<b>Inhibition of internalization of <i>Pseudomonas aeruginosa</i> strain PAO1 into transformed human WI-38 cells (diploid embryonic lung fibroblasts) and A549 cells (lung carcinoma cell line) by monoclonal antibodies (MAbs) and synthetic peptides specific to the first or fourth extracellular domains of CFTR</b> (Mean cfu (standard deviation) of <i>P. aeruginosa</i> internalized by the indicated cell line)		
Inhibitor added to assay	WI 38 cells	A549 cells
No inhibitor added	8.4733 X 10 <sup>4</sup> (5.111 X 10 <sup>3</sup> )	1.00067 X 10 <sup>5</sup> (6.088 X 10 <sup>3</sup> )
1nM Peptide to 4th Extracellular Domain	7.3433 X 10 <sup>4</sup> (9.124 X 10 <sup>3</sup> )	8.7333 X 10 <sup>4</sup> (1.1506 X 10 <sup>4</sup> )
1 nM Peptide of 1st Extracellular Domain	3.497 X 10 <sup>4</sup> (3.751 X 10 <sup>3</sup> ) <sup>a</sup>	3.2833 X 10 <sup>4</sup> (4.32 X 10 <sup>3</sup> ) <sup>a</sup>
MAb to 4th Extracellular Domain	6.6617 X 10 <sup>4</sup> (6.823 X 10 <sup>3</sup> )	8.7767 X 10 <sup>4</sup> (5.734 X 10 <sup>3</sup> )
MAb to 1st Extracellular Domain	2.26 X 10 <sup>4</sup> (5.166 X 10 <sup>3</sup> ) <sup>a</sup>	1.4833 X 10 <sup>4</sup> (4.142 X 10 <sup>3</sup> ) <sup>a</sup>

<sup>a</sup>, Significantly fewer internalized *P. aeruginosa* bacteria compared to corresponding reagent specific to the fourth extracellular domain of CFTR

**Example 3:** The first extracellular domain of CFTR binds to and inhibits internalization of *P. aeruginosa* in mouse lung

## Results:

To confirm that binding and internalization of *P. aeruginosa* by the first predicted extracellular domain of CFTR is important in resistance to lung infection, 7-day old Balb/c mice were nasally inoculated with  $10^8$  cfu of *P. aeruginosa* strain PAO1 mixed with either 10 nM of the peptide corresponding to the first or fourth predicted extracellular domains of CFTR and the course of bacterial infection was followed for over 24 hours [7, 16]. Twenty-four hours post-infection, mice inoculated with bacteria plus the first extracellular domain peptide had virtually no internalized *P. aeruginosa*, as determined by gentamicin-exclusion assays on single-cell suspensions of lungs (table 7), while mice receiving the bacteria along with the fourth extracellular domain peptide had a median of  $>10^4$  cfu of *P. aeruginosa* internalized per mg of lung tissue (Table 7). As a consequence of this inhibition of internalization, mice receiving bacteria plus the first extracellular domain peptide had a median of  $\sim 1.5 \times 10^5$  cfu of *P. aeruginosa* per mg of lung tissue as compared with a median of  $\sim 2 \times 10^4$  cfu *P. aeruginosa* per mg of lung tissue for animals infected with bacteria plus the fourth predicted extracellular domain (Table 7). Thus, inhibiting *P. aeruginosa* internalization by blocking the bacterial interaction with CFTR in the lung leads to increased bacterial counts in this tissue, indicating an important mechanism for clearance of *P. aeruginosa* from the lung following inhalation of these organisms.

**Table 7**

<b>Effect of addition of 10 nM of the synthetic peptides corresponding to the first or fourth extracellular domains of CFTR to <math>10^8</math> cfu of <i>P. aeruginosa</i> strain PAO1 on internalization by lung cells and total cfu of bacteria in the lungs 24 hours after intranasal infection of 7-day old mice.(Median cfu (10th - 90th percentile cfu) <i>P. aeruginosa</i>)</b>		
Peptide added	Internalized per mg. lung tissue	Total per mg. lung tissue
First extracellular domain	0 (0-278)	152,419 (37,860 -519,612)
Fourth extracellular domain	13,246 (3,578-49,558)	20,450 (4,000- 61,008)

**Example 4: Administration of LPS core enhances CFTR function in  $\Delta$ F508 cells without inhibiting ingestion of *P. Aeruginosa***

**Results:**

Although *P. aeruginosa* LPS core-oligosaccharide has been used in the past in ligand-mediated inhibition of epithelial cell ingestion to demonstrate the importance of this phenomenon in bacterial clearance from the lung [7] and to show that *P. aeruginosa* LPS core-oligosaccharide can inhibit the ingestion, experiments were carried out to determine whether it was possible to deliver the purified bacterial ligand via a route or dose that stimulates CFTR trafficking but is insufficient to inhibit bacterial clearance from the lung. To determine if CFTR trafficking can be stimulated by *P. aeruginosa* complete-core LPS oligosaccharide, an *in vitro* cellular uptake assay was used. The CFT1 and CFT1-F508 lines of transformed human airway epithelial cells were treated with either *P. aeruginosa* complete- or incomplete-core oligosaccharide for 24 hrs. prior to use of the cells in the standard bacterial uptake assay. Residual extracellular oligosaccharide is washed away prior to adding bacteria for evaluation of uptake. As shown in Table 8, significant stimulation (over 8-fold) of *P. aeruginosa* ingestion by the CFT1 cell line with 3 copies of the mutant  $\Delta$ F508-CFTR-gene (CFT1-F508 cells) was observed by incubating with complete-core oligosaccharide, whereas incomplete-core oligosaccharide resulted in no enhancement in uptake of *P. aeruginosa*. A comparable effect was observed using the parental CFT1 cell line with two copies of the  $\Delta$ F508-CFTR gene. Therefore, it was found that preincubation with a complete LPS core could enhance the function of  $\Delta$ F508 CFTR without inhibiting ingestion of *P. aeruginosa*.

Table 8

<b>Augmentation of ingestion of <i>P. aeruginosa</i> strain PAO1 by treatment of CFT1 or CFT1-F508 cells with complete- or incomplete-core oligosaccharide from <i>P. aeruginosa</i> strain PAC557.</b> <b>[Mean cfu (standard deviation) of <i>P. aeruginosa</i> internalized]</b>				
Amount (µg/ml)	Complete core oligosaccharide (CFT1 cells)	Incomplete core oligosaccharide (CFT1- ΔF508)	Complete core oligosaccharide (CFT1- ΔF508)	Incomplete core oligosaccharide (CFT1 cells)
100	6.16 X 10 <sup>4</sup> (1.15 X 10 <sup>4</sup> ) <sup>a</sup>	7.4 X 10 <sup>3</sup> (1.37 X 10 <sup>3</sup> )	9.48 X 10 <sup>4</sup> (3.84 X 10 <sup>4</sup> ) <sup>a</sup>	8.21 X 10 <sup>3</sup> (1.1 X 10 <sup>3</sup> )
50	6.04 X 10 <sup>4</sup> (1.47 X 10 <sup>4</sup> ) <sup>a</sup>	7.47 X 10 <sup>3</sup> (1.35 X 10 <sup>3</sup> )	9.12 X 10 <sup>4</sup> (4.59 X 10 <sup>3</sup> ) <sup>a</sup>	1.6 X 10 <sup>4</sup> (6.93 X 10 <sup>3</sup> )
25	4.55 X 10 <sup>4</sup> (8.27 X 10 <sup>3</sup> ) <sup>a</sup>	1.08 X 10 <sup>4</sup> (2.79 X 10 <sup>3</sup> )	4.69 X 10 <sup>4</sup> (6.64 X 10 <sup>3</sup> ) <sup>a</sup>	1.26 X 10 <sup>4</sup> (3.35 X 10 <sup>3</sup> )
10	2.69 X 10 <sup>4</sup> (4.4 X 10 <sup>3</sup> )	1.69 X 10 <sup>4</sup> (3.52 X 10 <sup>3</sup> )	2.1 X 10 <sup>4</sup> (2.05 X 10 <sup>3</sup> )	1.78 X 10 <sup>4</sup> (4.82 X 10 <sup>3</sup> )
1	2.53 X 10 <sup>4</sup> (5.82 X 10 <sup>3</sup> )	2.7 X 10 <sup>4</sup> (8.37 X 10 <sup>3</sup> )	2.11 X 10 <sup>4</sup> (3.72 X 10 <sup>3</sup> )	2.78 X 10 <sup>4</sup> (5.73 X 10 <sup>3</sup> )

<sup>a</sup>, Significantly more internalized *P. aeruginosa* bacteria compared to corresponding cell line treated with incomplete core oligosaccharide

**Example 5:** The LPS core enhances CFTR function *in vivo* by enhancing cellular uptake and clearance of *P. aeruginosa*

For the above *in vitro* finding to be of potential therapeutic value there was a need to demonstrate that treatment of an animal with the bacterial ligand for ingestion stimulates *P. aeruginosa* uptake *in vivo* and promotes bacterial clearance from the lung. The experimental approach used was quite different from that used to generate previously reported data [7] where it was shown that inclusion of the complete-core oligosaccharide ligand in the bacterial inoculum inhibited *P. aeruginosa* ingestion and promoted enhanced bacterial growth in the lungs of neonatal mice. The *in vitro* results (Table 8) suggest that purified ligand (a small molecular sized, nonimmunogenic carbohydrate) stimulates receptor trafficking and enhances *P. aeruginosa* uptake if cells are exposed to it prior to exposure to the bacterial inoculum. Treating

an individual with purified ligand could potentially enhance expression of the bacterial receptor that promotes epithelial cell ingestion, leading to greater clearance of bacteria *in vivo*. This was initially evaluated in mice by priming them with purified oligosaccharide 24 hrs. prior to bacterial challenge, using the model of Tang et al. [16].

To avoid any inhibitory effects of complete-core oligosaccharide on bacterial clearance, the oligosaccharide was administered intraperitoneally (IP) and bacterial clearance following lung challenge was monitored. Although it is recognized that clearance occurs by cellular binding of bacteria in the lumen of the airway, and it is also clearly recognized that CFTR, the ligand for *P. aeruginosa* ingestion, is located in the apical membrane, the initial experiments were performed using systemic therapy. It was reasoned that if enhanced bacterial uptake and clearance were obtained using this route, potential inhibitory complications from complete-core oligosaccharide in the airway lumen could be minimized. As shown in Table 9, IP injection of 100 µg of *P. aeruginosa* complete-core oligosaccharide resulted in significantly reduced levels of bacteria in the airways of neonatal mice 24 hrs. after nasal application of  $5 \times 10^7$  cfu of *P. aeruginosa* strain PAO1, as compared to mice primed with incomplete-core LPS oligosaccharide. In addition to measuring the bacterial load in the lungs, histopathologic examination of lungs of these mice showed that those receiving the complete-core oligosaccharide primer had only mildly affected tissues, whereas the incomplete-core oligosaccharide primed mice had extensive inflammation and damage, identical to that reported by Tang et al. [16] in mice infected for 24 hours by *P. aeruginosa* PAO1.

Table 9

Bacterial load in lungs of neonatal mice (24 hrs. after challenge) primed 24 hrs. prior to challenge with <i>P. aeruginosa</i> LPS-core oligosaccharides.	
Geometric mean cfu bacteria/lung (95% C.I.) in mice primed with <i>P. aeruginosa</i> complete- core LPS oligosaccharide 24 hrs. prior to bacterial challenge	Geometric mean cfu bacteria/lung (95% C.I.) in mice primed with <i>P. aeruginosa</i> incomplete-core LPS oligosaccharide 24 hrs. prior to bacterial challenge
1,980 (618-5353)	58,529 (19,593-153,945)

The mechanism by which the LPS core causes enhanced clearance of bacteria from the lungs is unknown. Possibly it stimulates production of an apical-membrane receptor for bacterial ingestion, like CFTR. Small amounts of oligosaccharide may get to the luminal

surface via systemic transport where they may bind to the epithelial cell receptor and stimulate production of more receptor, or there may be a way that adsorption of oligosaccharide from the basal side of the epithelial cells also stimulates receptor production. Alternately, several studies have suggested an intracellular function for CFTR [19-21] and other studies have demonstrated the presence of CFTR in endosomes [22] and clathrin-coated vesicles [23]. Thus, it is possible that entry of *P. aeruginosa* oligosaccharide from the basal side of the cell stimulates intracellular CFTR (or another membrane protein involved in internalization of *P. aeruginosa*) trafficking by binding to the intracellular CFTR (or other receptor).

Additional validation of these results was sought by repeating the experiment of priming mice with oligosaccharide injected IP 24 hours prior to nasal application of *P. aeruginosa*, but this time measuring bacterial uptake by the lung epithelial cells 4 hours after infection, using the gentamicin-survival assay. In this study mice primed with complete-core oligosaccharide has 3 to 4 times as many intracellular *P. aeruginosa* cells (mean  $280,870 \pm 30,180$ ) compared to mice primed with either nothing ( $82,200 \pm 2,660$ ) or incomplete-core oligosaccharide ( $67,980 \pm 4790$ ) ( $P < .001$ , ANOVA). Four-hours post infection mice primed with complete-core oligosaccharide had slightly (nonsignificant) lower total cfu per lung. Thus, as opposed to the results shown in fig. 4 of reference [7], where inclusion of the complete-core oligosaccharide with the bacterial inoculum inhibited cellular ingestion and promoted *P. aeruginosa* survival in the lungs of neonatal mice, priming mice with the same material 24 hours prior to infection stimulated bacterial uptake and clearance from the lungs, suggesting that epithelial-cell receptors had been up-regulated by the priming.

In conclusion, published results identified [7] the complete outer-core oligosaccharide portion of the *P. aeruginosa* lipopolysaccharide (LPS) as the bacterial ligand for internalization by human airway cells. Results here identify the first predicted extracellular domain of CFTR, encompassing amino acids 103-117 of the mature protein, as the cellular receptor, and this receptor can be up-regulated by pretreatment of either cells or animals with complete core-oligosaccharide derived from the *P. aeruginosa* LPS.

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Each of the foregoing patents, patent applications and references is herein incorporated by reference in its entirety. Having described the presently preferred embodiments, in accordance with the present invention, it is believed that other modifications, variations and changes will be suggested to those skilled in the art in view of the teachings set forth herein. It is, therefore, to be understood that all such variations, modifications, and changes are believed to fall within the scope of the present invention as defined by the appended claims.

## SEQUENCE LISTING

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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Gates, Edward R
- (B) REGISTRATION NUMBER: 31,616
- (C) REFERENCE/DOCKET NUMBER: B0801/7054

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617-720-3500
- (B) TELEFAX: 617-720-2441

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6129 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 133..4575

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTGGAAGC AAATGACATC ACAGCAGGTC AGAGAAAAAG GGTGAGCGG CAGGCACCCA

60

GAGTAGTAGG TCTTTGGCAT TAGGAGCTTG AGCCAGACG GCCCTAGCAG GGACCCAGC

120

GCCCGAGAGA CC ATG CAG AGG TCG CCT CTG GAA AAG GCC AGC GTT GTC

168

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val

1

5

10

TCC AAA CTT TTT TTC AGC TGG ACC AGA CCA ATT TTG AGG AAA GGA TAC

216

Ser Lys Leu Phe Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr

15

20

25

AGA CAG CGC CTG GAA TTG TCA GAC ATA TAC CAA ATC CCT TCT GTT GAT

264

Arg Gln Arg Leu Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp

30

35

40

TCT GCT GAC AAT CTA TCT GAA AAA TTG GAA AGA GAA TGG GAT AGA GAG

312

Ser Ala Asp Asn Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu

45

50

55

60

CTG GCT TCA AAG AAA AAT CCT AAA CTC ATT AAT GCC CTT CGG CGA TGT

360

Leu Ala Ser Lys Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys

65

70

75

TTT TTC TGG AGA TTT ATG TTC TAT GGA ATC TTT TTA TAA TTA GGG GAA

408

Phe Phe Trp Arg Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu

80

85

90

GTC ACC AAA GCA GTA CAG CCT CTC TTA CTG GGA AGA ATC ATA GCT TCC

456

Val Thr Lys Ala Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser

95

100

105

TAT GAC CCG GAT AAC AAG GAG GAA CGC TCT ATC GCG ATT TAT CTA GGC

504

Tyr Asp Pro Asp Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly

110

115

120

ATA GGC TTA TGC CTT CTC TTT ATT GTG AGG ACA CTG CTC CTA CAC CCA

552

Ile Gly Leu Cys Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro

125

130

135

140

GCC ATT TTT GGC CTT CAT CAC ATT GGA ATG CAG ATG AGA ATA GCT ATG

600

Ala Ile Phe Gly Leu His His Ile Gly Met Gln Met Arg Ile Ala Met

145

150

155

TTT AGT TTG ATT TAT AAG AAG ACT TTA AAG CTG TCA AGC CGT GTT CTA

648

Phe Ser Leu Ile Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu

160

165

170

GAT AAA ATA AGT ATT GGA CAA CTT GTT AGT CTC CTT TCC AAC AAC CTG

696

Asp Lys Ile Ser Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu  
 175 180 185  
 AAC AAA TTT GAT GAA GGA CTT GCA TTG GCA CAT TTC GTG TGG ATC GCT  
 744  
 Asn Lys Phe Asp Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala  
 190 195 200  
 CCT TTG CAA GTG GCA CTC CTC ATG GGG CTA ATC TGG GAG TTG TTA CAG  
 792  
 Pro Leu Gln Val Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln  
 205 210 215 220  
 GCG TCT GCC TTC TGT GGA CTT GGT TTC CTG ATA GTC CTT GCC CTT TTT  
 840  
 Ala Ser Ala Phe Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe  
 225 230 235  
 CAG GCT GGG CTA GGG AGA ATG ATG ATG AAG TAC AGA GAT CAG AGA GCT  
 888  
 Gln Ala Gly Leu Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala  
 240 245 250  
 GGG AAG ATC AGT GAA AGA CTT GTG ATT ACC TCA GAA ATG ATT GAA AAT  
 936  
 Gly Lys Ile Ser Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn  
 255 260 265  
 ATC CAA TCT GTT AAG GCA TAC TGC TGG GAA GAA GCA ATG GAA AAA ATG  
 984  
 Ile Gln Ser Val Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met  
 270 275 280

ATT GAA AAC TTA AGA CAA ACA GAA CTG AAA CTG ACT CGG AAG GCA GCC  
 1032  
 Ile Glu Asn Leu Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala  
 285 290 295 300

TAT GTG AGA TAC TTC AAT AGC TCA GCC TTC TTC TTC TCA GGG TTC TTT  
 1080  
 Tyr Val Arg Tyr Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe  
 305 310 315

GTG GTG TTT TTA TCT GTG CTT CCC TAT GCA CTA ATC AAA GGA ATC ATC  
 1128  
 Val Val Phe Leu Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile  
 320 325 330

CTC CGG AAA ATA TTC ACC ACC ATC TCA TTC TGC ATT GTT CTG CGC ATG  
 1176  
 Leu Arg Lys Ile Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met  
 335 340 345

GCG GTC ACT CGG CAA TTT CCC TGG GCT GTA CAA ACA TGG TAT GAC TCT  
 1224  
 Ala Val Thr Arg Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser  
 350 355 360

CTT GGA GCA ATA AAC AAA ATA CAG GAT TTC TTA CAA AAG CAA GAA TAT  
 1272  
 Leu Gly Ala Ile Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr  
 365 370 375 380

AAG ACA TTG GAA TAT AAC TTA ACG ACT ACA GAA GTA GTG ATG GAG AAT  
 1320  
 Lys Thr Leu Glu Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn



GTA ACA GCC TTC TGG GAG GAG GGA TTT GGG GAA TTA TTT GAG AAA GCA  
1368

Val Thr Ala Phe Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala  
400 405 410

AAA CAA AAC AAT AAC AAT AGA AAA ACT TCT AAT GGT GAT GAC AGC CTC  
1416

Lys Gln Asn Asn Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu  
415 420 425

TTC TTC AGT AAT TTC TCA CTT CTT GGT ACT CCT GTC CTG AAA GAT ATT  
1464

Phe Phe Ser Asn Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile  
430 435 440

AAT TTC AAG ATA GAA AGA GGA CAG TTG TTG GCG GTT GCT GGA TCC ACT  
1512

Asn Phe Lys Ile Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr  
445 450 455 460

GGA GCA GGC AAG ACT TCA CTT CTA ATG ATG ATT ATG GGA GAA CTG GAG  
1560

Gly Ala Gly Lys Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu  
465 470 475

CCT TCA GAG GGT AAA ATT AAG CAC AGT GGA AGA ATT TCA TTC TGT TCT  
1608

Pro Ser Glu Gly Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser  
480 485 490

CAG TTT TCC TGG ATT ATG CCT GGC ACC ATT AAA GAA AA. ATC ATC TTT  
1656

Gln Phe Ser Trp Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe  
495 500 505

GGT GTT TCC TAT GAT GAA TAT AGA TAC AGA AGC GTC ATC AAA GCA TGC  
1704

Gly Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys  
510 515 520

CAA CTA GAA GAG GAC ATC TCC AAG TTT GCA GAG AAA GAC AAT ATA GTT  
1752

Gln Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val  
525 530 535 540

CTT GGA GAA GGT GGA ATC ACA CTG AGT GGA GGT CAA CGA GCA AGA ATT  
1800

Leu Gly Glu Gly Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile  
545 550 555

TCT TTA GCA AGA GCA GTA TAC AAA GAT GCT GAT TTG TAT TTA TTA GAC  
1848

Ser Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp  
560 565 570

TCT CCT TTT GGA TAC CTA GAT GTT TTA ACA GAA AAA GAA ATA TTT GAA  
1896

Ser Pro Phe Gly Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu  
575 580 585

AGC TGT GTC TGT AAA CTG ATG GCT AAC AAA ACT AGG ATT TTG GTC ACT  
1944

Ser Cys Val Cys Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr

590

595

600

TCT AAA ATG GAA CAT TTA AAG AAA GCT GAC AAA ATA TTA ATT TTG AAT  
1992

Ser Lys Met Glu His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn  
605 610 615 620

GAA GGT AGC AGC TAT TTT TAT GGG ACA TTT TCA GAA CTC CAA AAT CTA  
2040

Glu Gly Ser Ser Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu  
625 630 635

CAG CCA GAC TTT AGC TCA AAA CTC ATG GGA TGT GAT TCT TTC GAC CAA  
2088

Gln Pro Asp Phe Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln  
640 645 650

TTT AGT GCA GAA AGA AGA AAT TCA ATC CTA ACT GAG ACC TTA CAC CGT  
2136

Phe Ser Ala Glu Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg  
655 660 665

TTC TCA TTA GAA GGA GAT GCT CCT GTC TCC TGG ACA GAA ACA AAA AAA  
2184

Phe Ser Leu Glu Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys  
670 675 680

CAA TCT TTT AAA CAG ACT GGA GAG TTT GGG GAA AAA AGG AAG AAT TCT  
2232

Gln Ser Phe Lys Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser  
685 690 695 700

ATT CTC AAT CCA ATC AAC TCT ATA CGA AAA TTT TCC AAT GTG CAA AAG  
2280

Ile Leu Asn Pro Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys  
705 710 715

ACT CCC TTA CAA ATG AAT GGC ATC GAA GAG GAT TCT GAT GAG CCT TTA  
2328

Thr Pro Leu Gln Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu  
720 725 730

GAG AGA AGG CTG TCC TTA GTA CCA GAT TCT GAG CAG GGA GAG GCG ATA  
2376

Glu Arg Arg Leu Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile  
735 740 745

CTG CCT CGC ATC AGC GTG ATC AGC ACT GGC CCC ACG CTT CAG GCA CGA  
2424

Leu Pro Arg Ile Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg  
750 755 760

AGG AGG CAG TCT GTC CTG AAC CTG ATG ACA CAC TCA GTT AAC CAA GGT  
2472

Arg Arg Gln Ser Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly  
765 770 775 780

CAG AAC ATT CAC CGA AAG ACA ACA GCA TCC ACA CGA AAA GTG TCA CTG  
2520

Gln Asn Ile His Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu  
785 790 795

GCC CCT CAG GCA AAC TTG ACT GAA CTG GAT ATA TAT TCA AGA AGG TTA  
2568

Ala Pro Gln Ala Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu

TCT CAA GAA ACT GGC TTG GAA ATA AGT GAA GAA ATT AAC GAA GAA GAC  
2616

Ser Gln Glu Thr Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp  
815 820 825

TTA AAG GAG TGC CTT TTT GAT GAT ATG GAG AGC ATA CCA GCA GTG ACT  
2664

Leu Lys Glu Cys Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr  
830 835 840

ACA TGG AAC ACA TAC CTT CGA TAT ATT ACT GTC CAC AAG AGC TTA ATT  
2712

Thr Trp Asn Thr Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile  
845 850 855 860

TTT GTG CTA ATT TGG TGC TTA GTA ATT TTT CTG GCA GAG GTG GCT GCT  
2760

Phe Val Leu Ile Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala  
865 870 875

TCT TTG GTT GTG CTG TGG CTC CTT GGA AAC ACT CCT CTT CAA GAC AAA  
2808

Ser Leu Val Val Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys  
880 885 890

GGG AAT AGT ACT CAT AGT AGA AAT AAC AGC TAT GCA GTG ATT ATC ACC  
2856

Gly Asn Ser Thr His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr  
895 900 905

AGC ACC AGT TCG TAT TAT GTG TTT TAC ATT TAC GTG GGA GTA GCC GAC

2904

Ser Thr Ser Ser Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp

910

915

920

ACT TTG CTT GCT ATG GGA TTC TTC AGA GGT CTA CCA CTG GTG CAT ACT

2952

Thr Leu Leu Ala Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr

925

930

935

940

CTA ATC ACA GTG TCG AAA ATT TTA CAC CAC AAA ATG TTA CAT TCT GTT

3000

Leu Ile Thr Val Ser Lys Ile Leu His His Lys Met Leu His Ser Val

945

950

955

CTT CAA GCA CCT ATG TCA ACC CTC AAC ACG TTG AAA GCA GGT GGG ATT

3048

Leu Gln Ala Pro Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile

960

965

970

CTT AAT AGA TTC TCC AAA GAT ATA GCA ATT TTG GAT GAC CTT CTG CCT

3096

Leu Asn Arg Phe Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro

975

980

985

CTT ACC ATA TTT GAC TTC ATC CAG TTG TTA TTA ATT GTG ATT GGA GCT

3144

Leu Thr Ile Phe Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala

990

995

1000

ATA GCA GTT GTC GCA GTT TTA CAA CCC TAC ATC TTT GTT GCA ACA GTG

3192

Ile Ala Val Val Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val

1005 1010 1015 1020

CCA GTG ATA GTG GCT TTT ATT ATG TTG AGA GCA TAT TTC CTC CAA ACC  
3240

Pro Val Ile Val Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr  
1025 1030 1035

TCA CAG CAA CTC AAA CAA CTG GAA TCT GAA GGC AGG AGT CCA ATT TTC  
3288

Ser Gln Gln Leu Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe  
1040 1045 1050

ACT CAT CTT GTT ACA AGC TTA AAA GGA CTA TGG ACA CTT CGT GCC TTC  
3336

Thr His Leu Val Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe  
1055 1060 1065

GGA CGG CAG CCT TAC TTT GAA ACT CTG TTC CAC AAA GCT CTG AAT TTA  
3384

Gly Arg Gln Pro Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu  
1070 1075 1080

CAT ACT GCC AAC TGG TTC TTG TAC CTG TCA ACA CTG CGC TGG TTC CAA  
3432

His Thr Ala Asn Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln  
1085 1090 1095 1100

ATG AGA ATA GAA ATG ATT TTT GTC ATC TTC TTC ATT GCT GTT ACC TTC  
3480

Met Arg Ile Glu Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe  
1105 1110 1115

ATT TCC ATT TTA ACA ACA GGA GAA GGA GAA GGA AGA G1. GGT ATT ATC  
3528

Ile Ser Ile Leu Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile  
1120 1125 1130

CTG ACT TTA GCC ATG AAT ATC ATG AGT ACA TTG CAG TGG GCT GTA AAC  
3576

Leu Thr Leu Ala Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn  
1135 1140 1145

TCC AGC ATA GAT GTG GAT AGC TTG ATG CGA TCT GTG AGC CGA GTC TTT  
3624

Ser Ser Ile Asp Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe  
1150 1155 1160

AAG TTC ATT GAC ATG CCA ACA GAA GGT AAA CCT ACC AAG TCA ACC AAA  
3672

Lys Phe Ile Asp Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys  
1165 1170 1175 1180

CCA TAC AAG AAT GGC CAA CTC TCG AAA GTT ATG ATT ATT GAG AAT TCA  
3720

Pro Tyr Lys Asn Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser  
1185 1190 1195

CAC GTG AAG AAA GAT GAC ATC TGG CCC TCA GGG GGC CAA ATG ACT GTC  
3768

His Val Lys Lys Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val  
1200 1205 1210

AAA GAT CTC ACA GCA AAA TAC ACA GAA GGT GGA AAT GCC ATA TTA GAG  
3816

Lys Asp Leu Thr Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu



1215

1220

1225

AAC ATT TCC TTC TCA ATA AGT CCT GGC CAG AGG GTG GGC CTC TTG GGA  
3864

Asn Ile Ser Phe Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly  
1230 1235 1240

AGA ACT GGA TCA GGG AAG AGT ACT TTG TTA TCA GCT TTT TTG AGA CTA  
3912

Arg Thr Gly Ser Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu  
1245 1250 1255 1260

CTG AAC ACT GAA GGA GAA ATC CAG ATC GAT GGT GTG TCT TGG GAT TCA  
3960

Leu Asn Thr Glu Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser  
1265 1270 1275

ATA ACT TTG CAA CAG TGG AGG AAA GCC TTT GGA GTG ATA CCA CAG AAA  
4008

Ile Thr Leu Gln Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys  
1280 1285 1290

GTA TTT ATT TTT TCT GGA ACA TTT AGA AAA AAC TTG GAT CCC TAT GAA  
4056

Val Phe Ile Phe Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu  
1295 1300 1305

CAG TGG AGT GAT CAA GAA ATA TGG AAA GTT GCA GAT GAG GTT GGG CTC  
4104

Gln Trp Ser Asp Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu  
1310 1315 1320

AGA TCT GTG ATA GAA CAG TTT CCT GGG AAG CTT GAC TTT GTC CTT GTG

4152

Arg Ser Val Ile Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val

1325

1330

1335

1340

GAT GGG GGC TGT GTC CTA AGC CAT GGC CAC AAG CAG TTG ATG TGC TTG

4200

Asp Gly Gly Cys Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu

1345

1350

1355

GCT AGA TCT GTT CTC AGT AAG GCG AAG ATC TTG CTG CTT GAT GAA CCC

4248

Ala Arg Ser Val Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro

1360

1365

1370

AGT GCT CAT TTG GAT CCA GTA ACA TAC CAA ATA ATT AGA AGA ACT CTA

4296

Ser Ala His Leu Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu

1375

1380

1385

AAA CAA GCA TTT GCT GAT TGC ACA GTA ATT CTC TGT GAA CAC AGG ATA

4344

Lys Gln Ala Phe Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile

1390

1395

1400

GAA GCA ATG CTG GAA TGC CAA CAA TTT TTG GTC ATA GAA GAG AAC AAA

4392

Glu Ala Met Leu Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys

1405

1410

1415

1420

GTG CGG CAG TAC GAT TCC ATC CAG AAA CTG CTG AAC GAG AGG AGC CTC

4440

Val Arg Gln Tyr Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu

TTC CGG CAA GCC ATC AGC CCC TCC GAC AGG GTG AAG CTC TTT CCC CAC  
4488

Phe Arg Gln Ala Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His  
1440 1445 1450

CGG AAC TCA AGC AAG TGC AAG TCT AAG CCC CAG ATT GCT GCT CTG AAA  
4536

Arg Asn Ser Ser Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys  
1455 1460 1465

GAG GAG ACA GAA GAA GAG GTG CAA GAT ACA AGG CTT TAGAGAGCAG  
4582

Glu Glu Thr Glu Glu Glu Val Gln Asp Thr Arg Leu  
1470 1475 1480

CATAAATGTT GACATGGGAC ATTTGCTCAT GGAATTGGAG CTCGTGGGAC AGTCACCTCA  
4642

TGGAATTGGA GCTCGTGGAA CAGTTACCTC TGCCTCAGAA AACAAGGATG AATTAAGTTT  
4702

TTTTTTAAAA AAGAAACATT TGGTAAGGGG AATTGAGGAC ACTGATATGG GTCTTGATAA  
4762

ATGGCTTCCT GGCAATAGTC AAATTGTGTG AAAGGTACTT CAAATCCTTG AAGATTTACC  
4822

ACTTGTGTTT TGCAAGCCAG ATTTTCCTGA AAACCCTTGC CATGTGCTAG TAATTGGAAA  
4882

GGCAGCTCTA AATGTCAATC AGCCTAGTTG ATCAGCTTAT TGTCTAGTGA AACTCGTTAA  
4942

TTTGTAGTGT TGGAGAAGAA CTGAAATCAT ACTTCTTAGG GTTATGATTA AGTAATGATA  
5002

ACTGGAAACT TCAGCGGTTT ATATAAGCTT GTATTCCTTT TTCTCTCCTC TCCCCATGAT  
5062

GTTTAGAAAC ACAACTATAT TGTTTGCTAA GCATTCCAAC TATCTCATTT CCAAGCAAGT  
5122

ATTAGAATAC CACAGGAACC ACAAGACTGC ACATCAAAAT ATGCCCCATT CAACATCTAG  
5182

TGAGCAGTCA GGAAAGAGAA CTTCCAGATC CTGGAAATCA GGGTTAGTAT TGTCCAGGTC  
5242

TACCAAAAAT CTCAATATTT CAGATAATCA CAATACATCC CTTACCTGGG AAAGGGCTGT  
5302

TATAATCTTT CACAGGGGAC AGGATGGTTC CCTTGATGAA GAAGTTGATA TGCCTTTTCC  
5362

CAACTCCAGA AAGTGACAAG CTCACAGACC TTTGAACTAG AGTTTAGCTG GAAAAGTATG  
5422

TTAGTGCAAA TTGTCACAGG ACAGCCCTTC TTTCCACAGA AGCTCCAGGT AGAGGGTGTG  
5482

TAAGTAGATA GGCCATGGGC ACTGTGGGTA GACACACATG AAGTCCAAGC ATTTAGATGT  
5542

ATAGGTTGAT GGTGGTATG1 TTTCAGGCTA GATGTATGTA CTTCA1GCTG TCTACACTAA

5602

GAGAGAATGA GAGACACACT GAAGAAGCAC CAATCATGAA TTAGTTTTAT ATGCTTCTGT

5662

TTTATAATTT TGTGAAGCAA AATTTTTTCT CTAGGAAATA TTTATTTTAA TAATGTTTCA

5722

AACATATATT ACAATGCTGT ATTTTAAAAG AATGATTATG AATTACATTT GTATAAAATA

5782

ATTTTTATAT TTGAAATATT GACTTTTTAT GGCAC TAGTA TTTTATGAA ATATTATGTT

5842

AAAACTGGGA CAGGGGAGAA CCTAGGGTGA TATTAACCAG GGGCCATGAA TCACCTTTTG

5902

GTCTGGAGGG AAGCCTTGGG GCTGATCGAG TTGTTGCCCA CAGCTGTATG ATTCCCAGCC

5962

AGACACAGCC TCTTAGATGC AGTTCTGAAG AAGATGGTAC CACCAGTCTG ACTGTTTCCA

6022

TCAAGGGTAC ACTGCCTTCT CAACTCCAAA CTGACTCTTA AGAAGACTGC ATTATATTTA

6082

TTACTGTAAG AAAATATCAC TTGTCAATAA AATCCATACA TTTGTGT

6129

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1480 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe  
1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu  
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn  
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys  
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg  
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala  
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp  
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys  
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly  
 130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile  
 145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser  
 165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp  
 180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val  
 195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe  
 210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu  
 225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser  
 245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val  
 260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu  
 275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr  
 290 295 300

Phe	Asn	Ser	Ser	Ala	Phe	Phe	Ser	Gly	Phe	Phe	Val	Val	Phe	Leu
305					310				315					320

Ser	Val	Leu	Pro	Tyr	Ala	Leu	Ile	Lys	Gly	Ile	Ile	Leu	Arg	Lys	Ile
					325				330					335	

Phe	Thr	Thr	Ile	Ser	Phe	Cys	Ile	Val	Leu	Arg	Met	Ala	Val	Thr	Arg
					340				345					350	

Gln	Phe	Pro	Trp	Ala	Val	Gln	Thr	Trp	Tyr	Asp	Ser	Leu	Gly	Ala	Ile
					355				360					365	

Asn	Lys	Ile	Gln	Asp	Phe	Leu	Gln	Lys	Gln	Glu	Tyr	Lys	Thr	Leu	Glu
					370				375					380	

Tyr	Asn	Leu	Thr	Thr	Thr	Glu	Val	Val	Met	Glu	Asn	Val	Thr	Ala	Phe
385						390				395					400

Trp	Glu	Glu	Gly	Phe	Gly	Glu	Leu	Phe	Glu	Lys	Ala	Lys	Gln	Asn	Asn
					405				410					415	

Asn	Asn	Arg	Lys	Thr	Ser	Asn	Gly	Asp	Asp	Ser	Leu	Phe	Phe	Ser	Asn
					420				425					430	

Phe	Ser	Leu	Leu	Gly	Thr	Pro	Val	Leu	Lys	Asp	Ile	Asn	Phe	Lys	Ile
					435				440					445	

Glu	Arg	Gly	Gln	Leu	Leu	Ala	Val	Ala	Gly	Ser	Thr	Gly	Ala	Gly	Lys
					450				455					460	

Thr	Ser	Leu	Leu	Met	Met	Ile	Met	Gly	Glu	Leu	Glu	Pro	Ser	Glu	Gly
465						470						475			480



Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp

485

490

495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr

500

505

510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu

515

520

525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly

530

535

540

Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg

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560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly

565

570

575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys

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585

590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu

595

600

605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser

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620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe

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Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu

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655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu			
660	665	670	
Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys			
675	680	685	
Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro			
690	695	700	
Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln			
705	710	715	720
Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu			
725	730	735	
Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile			
740	745	750	
Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser			
755	760	765	
Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His			
770	775	780	
Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala			
785	790	795	800
Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr			
805	810	815	
Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys			
820	825	830	

Leu Phe Asp Asp Met Leu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr	835	840	845
Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile	850	855	860
Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val	865	870	875 880
Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr	885	890	895
His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser	900	905	910
Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala	915	920	925
Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val	930	935	940
Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro	945	950	955 960
Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe	965	970	975
Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe	980	985	990
Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val	995	1000	1005

Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val

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Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu

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Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val

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Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro

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1070

Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn

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1085

Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu

1090

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1100

Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu

1105

1110

1115

1120

Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala

1125

1130

1135

Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp

1140

1145

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Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp

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Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn

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Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys			
1185	1190	1195	1200
Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr			
	1205	1210	1215
Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe			
	1220	1225	1230
Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser			
	1235	1240	1245
Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu			
	1250	1255	1260
Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln			
1265	1270	1275	1280
Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe			
	1285	1290	1295
Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp			
	1300	1305	1310
Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile			
	1315	1320	1325
Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys			
	1330	1335	1340
Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val			
1345	1350	1355	1360

Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu

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Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe

1380

1385

1390

Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu

1395

1400

1405

Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr

1410

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Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala

1425

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Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser

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Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu

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Glu Glu Val Gln Asp Thr Arg Leu

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1480 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe  
1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu  
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn  
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys  
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg  
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala  
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp  
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys  
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Phe Ala Ile Phe Gly  
 130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile  
 145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser  
 165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp  
 180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val  
 195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe  
 210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu  
 225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser  
 245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val  
 260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu  
 275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr  
 290 295 300



Phe	Asn	Ser	Ser	Ala	Phe	Phe	Phe	Ser	Gly	Phe	Phe	Val	Val	Phe	Leu
305					310					315					320
Ser	Val	Leu	Pro	Tyr	Ala	Leu	Ile	Lys	Gly	Ile	Ile	Leu	Arg	Lys	Ile
					325					330					335
Phe	Thr	Thr	Ile	Ser	Phe	Cys	Ile	Val	Leu	Arg	Met	Ala	Val	Thr	Arg
					340					345					350
Gln	Phe	Pro	Trp	Ala	Val	Gln	Thr	Trp	Tyr	Asp	Ser	Leu	Gly	Ala	Ile
					355										360
Asn	Lys	Ile	Gln	Asp	Phe	Leu	Gln	Lys	Gln	Glu	Tyr	Lys	Thr	Leu	Glu
					370										380
Tyr	Asn	Leu	Thr	Thr	Thr	Glu	Val	Val	Met	Glu	Asn	Val	Thr	Ala	Phe
						385									400
Trp	Glu	Glu	Gly	Phe	Gly	Glu	Leu	Phe	Glu	Lys	Ala	Lys	Gln	Asn	Asn
						405									410
Asn	Asn	Arg	Lys	Thr	Ser	Asn	Gly	Asp	Asp	Ser	Leu	Phe	Phe	Ser	Asn
						420									430
Phe	Ser	Leu	Leu	Gly	Thr	Pro	Val	Leu	Lys	Asp	Ile	Asn	Phe	Lys	Ile
						435									440
Glu	Arg	Gly	Gln	Leu	Leu	Ala	Val	Ala	Gly	Ser	Thr	Gly	Ala	Gly	Lys
						450									460
Thr	Ser	Leu	Leu	Met	Met	Ile	Met	Gly	Glu	Leu	Glu	Pro	Ser	Glu	Gly
						465									480







Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val

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Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu

1025

1030

1035

1040

Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val

1045

1050

1055

Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro

1060

1065

1070

Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn

1075

1080

1085

Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu

1090

1095

1100

Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu

1105

1110

1115

1120

Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala

1125

1130

1135

Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp

1140

1145

1150

Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp

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Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn

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Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys			
1185	1190	1195	1200
Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr			
	1205	1210	1215
Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe			
	1220	1225	1230
Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser			
	1235	1240	1245
Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu			
	1250	1255	1260
Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln			
1265	1270	1275	1280
Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe			
	1285	1290	1295
Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp			
	1300	1305	1310
Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile			
	1315	1320	1325
Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys			
	1330	1335	1340
Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val			
1345	1350	1355	1360

Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu

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Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe

1380

1385

1390

Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu

1395

1400

1405

Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr

1410

1415

1420

Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala

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Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser

1445

1450

1455

Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu

1460

1465

1470

Glu Glu Val Gln Asp Thr Arg Leu

1475

1480

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly	Arg	Ile	Ile	Ala	Ser	Tyr	Asp	Pro	Asp	Asn	Lys	Glu	Glu	Arg
1				5				10					15	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr  
1 5 10 15

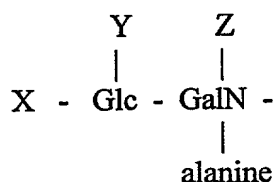
His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser  
20 25 30

What I claim is:

62014.1 79

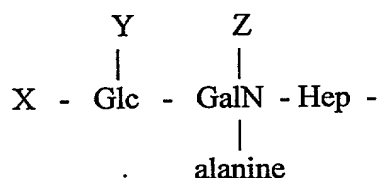
## Claims

1. A method for upregulating CFTR expression in a tissue of a subject comprising administering to a subject in need of such upregulation of CFTR expression a CFTR expression regulator in an amount effective to increase CFTR expression in said tissue of the subject, wherein the CFTR expression regulator is an isolated polysaccharide that is an LPS core moiety comprising

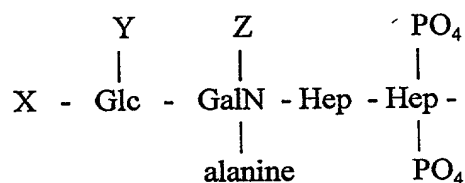


wherein X is selected from the group consisting of glucose, glucose-rhamnose and H; Y is selected from the group consisting of rhamnose and H; and Z is selected from the group consisting of glucose and H.

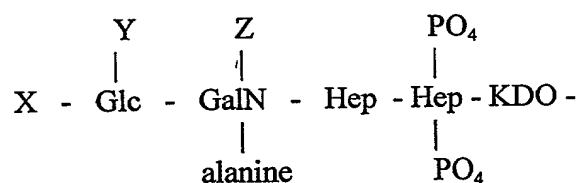
2. The method of claim 1 wherein the polysaccharide comprises



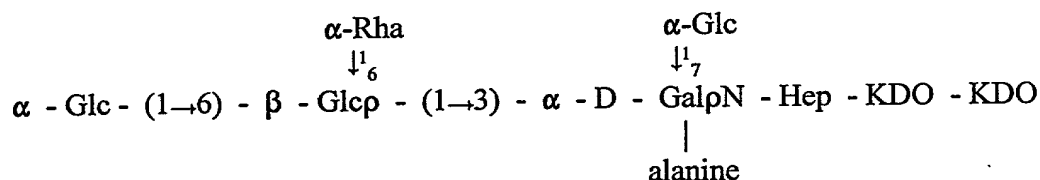
3. The method of claim 1 wherein the polysaccharide comprises



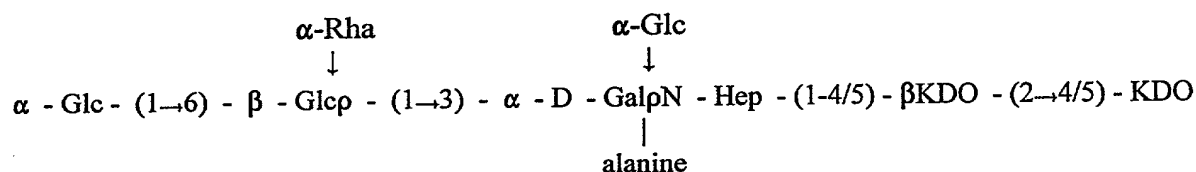
4. The method of claim 1 wherein the polysaccharide comprises



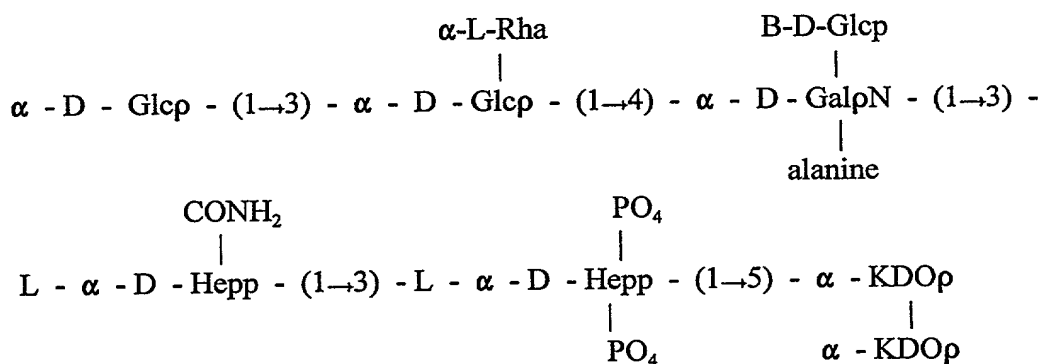
5. The method of claim 1 wherein the polysaccharide comprises



6. The method of claim 1 wherein the polysaccharide comprises



7. The method of claim 1 wherein the polysaccharide comprises



8. The method of claim 1 wherein the polysaccharide is a CFTR receptor-binding fragment of a lipopolysaccharide of *Pseudomonas aeruginosa*.

9. The method of claims 1-8 wherein the subject has a condition predisposing the subject to a *Pseudomonas* infection.

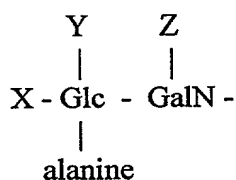
10. The method of claims 1-8 wherein the subject has a *Pseudomonas* infection.

11. The method of claims 1-8 wherein the subject has a defective cystic fibrosis transmembrane conductance regulator gene.

12. The method of claims 1-8 wherein the polysaccharide is administered systemically to the subject.

13. The method of claims 1-8 wherein the polysaccharide is administered by inhalation to the subject.

14. A pharmaceutical preparation comprising  
a therapeutically effective amount of a CFTR expression regulator, wherein the CFTR expression regulator is a polysaccharide that is an LPS core moiety comprising



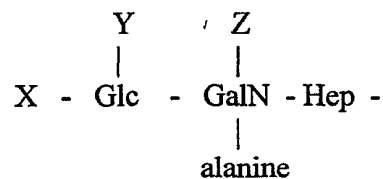
wherein X is selected from the group consisting of glucose, glucose-rhamnose and H;

wherein Y is selected from the group consisting of rhamnose and H; and

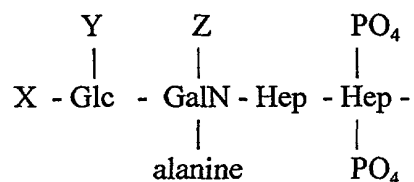
wherein Z is selected from the group consisting of glucose and H; and

a pharmaceutically acceptable carrier.

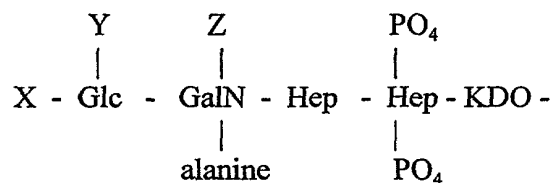
15. The pharmaceutical preparation of claim 14 wherein the polysaccharide comprises



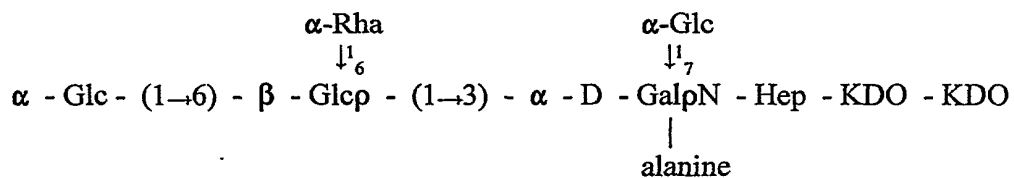
16. The pharmaceutical preparation of claim 14 wherein the polysaccharide comprises

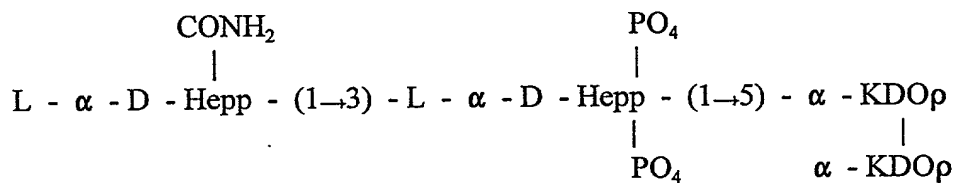


17. The pharmaceutical preparation of claim 14 wherein the polysaccharide comprises



18. The pharmaceutical preparation of claim 14 wherein the polysaccharide comprises



$$\begin{array}{ccccccc} & & \alpha\text{-Rha} & & \alpha\text{-Glc} & & \\ & & \downarrow & & \downarrow & & \\ \alpha\text{-Glc} - (1\rightarrow6) - \beta\text{-GlcP} - (1\rightarrow3) - \alpha\text{-D-GalpN} - \text{Hep} - (1\rightarrow4/5) - \beta\text{KDO} - (2\rightarrow4/5) - & & & & & & \\ \text{KDO} & & & & \text{alanine} & & \end{array}$$
$$\alpha\text{-D-Glcp} - (1\rightarrow3) - \alpha\text{-D-Glcp} - (1\rightarrow4) - \alpha\text{-D-GalpN} - (1\rightarrow3) - \text{alanine}$$


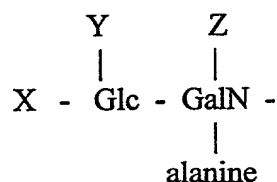
22. The pharmaceutical preparation of claims 14-21 wherein the pharmaceutical preparation is sterile.

62014.1

24. The pharmaceutical preparation of claims 14-21 wherein the pharmaceutical preparation is formulated as an aerosol for inhalation.

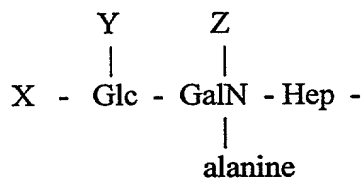
25. The pharmaceutical preparation of claims 14-21 wherein the pharmaceutical preparation is formulated as an injectable preparation.

26. A composition of matter comprising  
a covalent conjugate of a lipid biocompatible with a human subject and a polysaccharide comprising

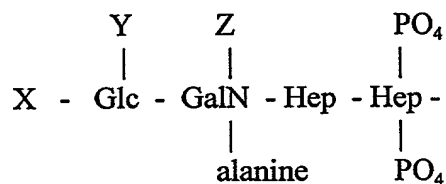


wherein X is selected from the group consisting of glucose, glucose-rhamnose and H; Y is selected from the group consisting of rhamnose and H; and Z is selected from the group consisting of glucose and H.

27. The composition of matter of claim 26 wherein the polysaccharide comprises

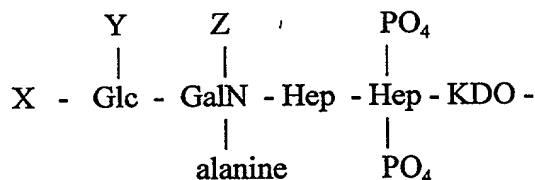


28. The composition of matter of claim 26 wherein the polysaccharide comprises

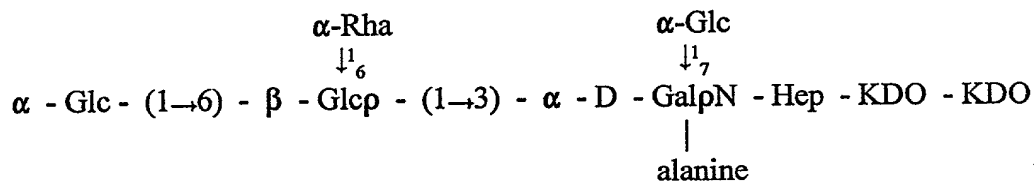




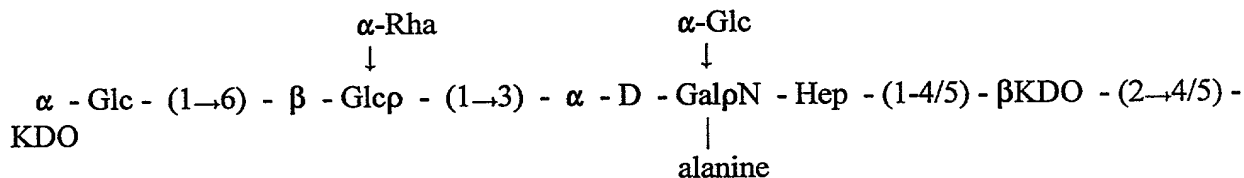
29. The composition of matter of claim 26 wherein the polysaccharide comprises



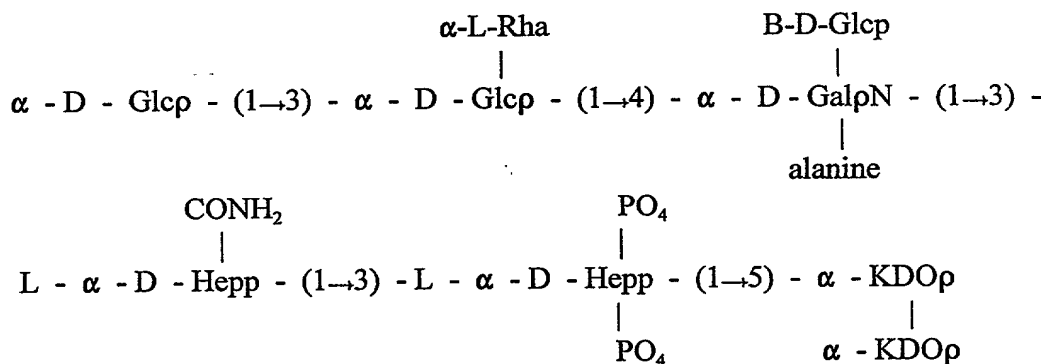
30. The composition of matter of claim 26 wherein the polysaccharide comprises



31. The composition of matter of claim 26 wherein the polysaccharide comprises



32. The composition of matter of claim 26 wherein the polysaccharide comprises

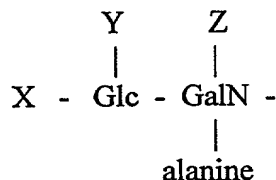


33. The composition of matter of claim 26 wherein the polysaccharide comprises a CFTR binding fragment of a lipopolysaccharide of *Pseudomonas aeruginosa*.

34. The composition of matter of claims 26-33 wherein the lipid has the following structural formula:  $\text{CH}_3(\text{CH}_2)_n\text{COOH}$  wherein  $n=1-50$ .

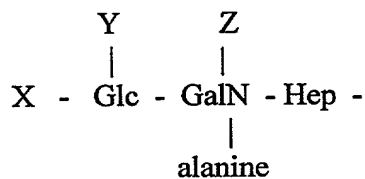
35. The composition of matter of claim 34 wherein the lipid wherein the lipid is in the wall of a liposome containing a bioactive agent.

36. A composition of matter comprising  
a covalent conjugate of a bioactive agent and a polysaccharide comprising

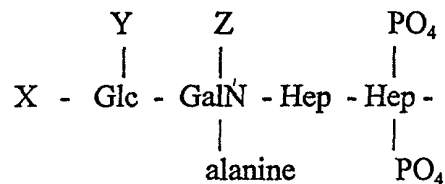


wherein X is selected from the group consisting of glucose, glucose-rhamnose and H; Y is selected from the group consisting of rhamnose and H; and Z is selected from the group consisting of glucose and H.

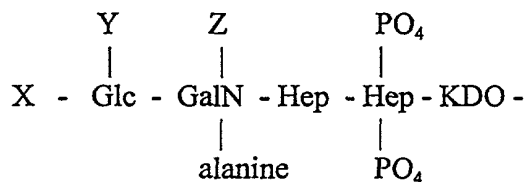
37. The composition of matter of claim 36 wherein the polysaccharide comprises



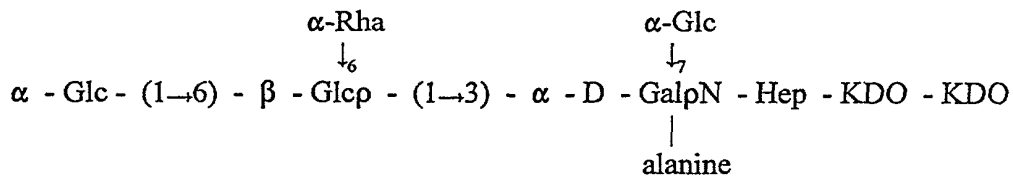
38. The composition of matter of claim 36 wherein the polysaccharide comprises



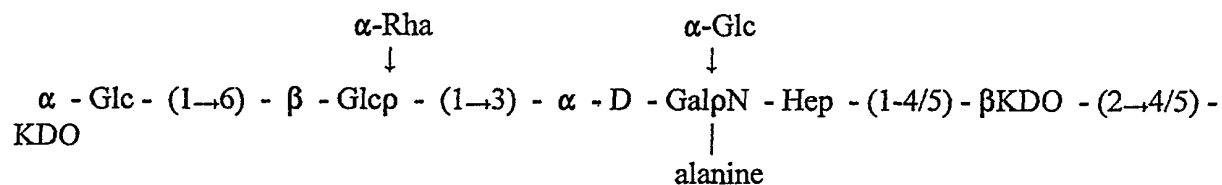
39. The composition of matter of claim 36 wherein the polysaccharide comprises



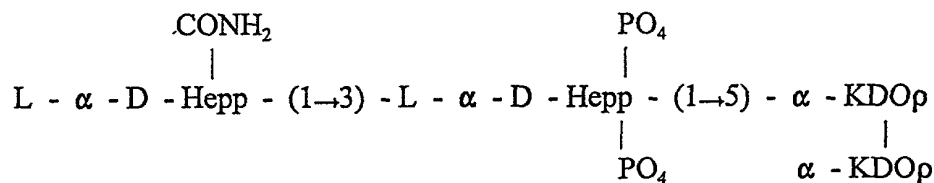
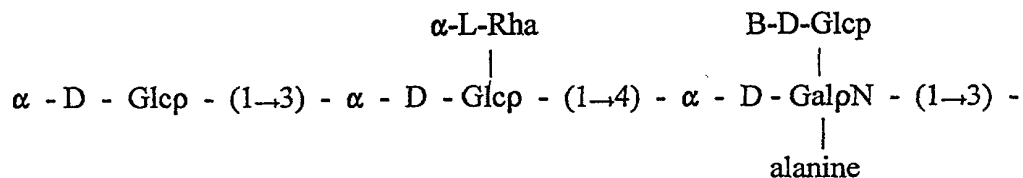
40. The composition of matter of claim 36 wherein the polysaccharide comprises



41. The composition of matter of claim 36 wherein the polysaccharide comprises



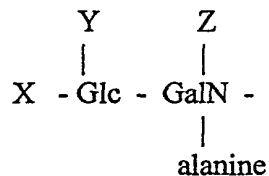
42. The composition of matter of claim 36 wherein the polysaccharide comprises



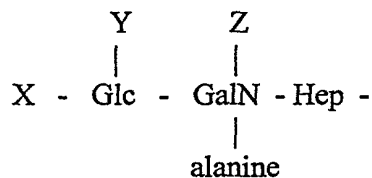
43. The composition of matter of claim 36 wherein the polysaccharide comprises a CFTR binding fragment of a lipopolysaccharide of *Pseudomonas aeruginosa*.

44. A method for delivering a bioactive agent to a tissue expressing a cystic fibrosis transmembrane regulator to treat a condition susceptible to treatment by said bioactive agent comprising

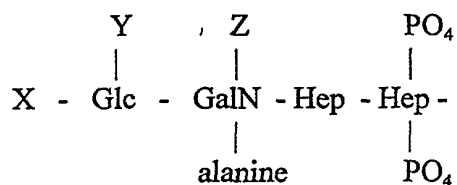
administering to a subject in need of such treatments said bioactive agent coupled to a polysaccharide comprising



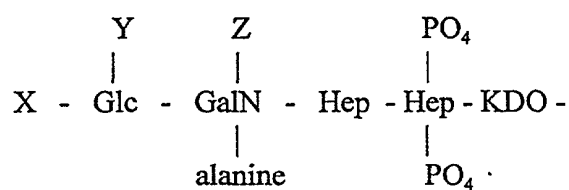
45. The method of claim 44, wherein the polysaccharide comprises



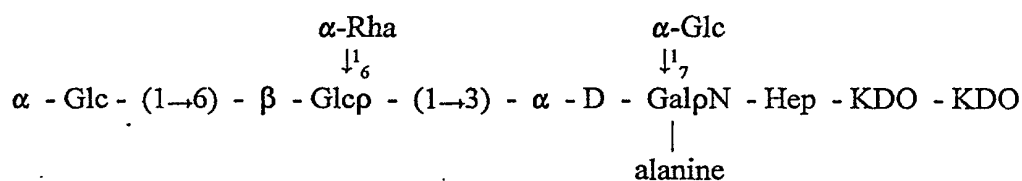
46. The method of claim 44, wherein the polysaccharide comprises



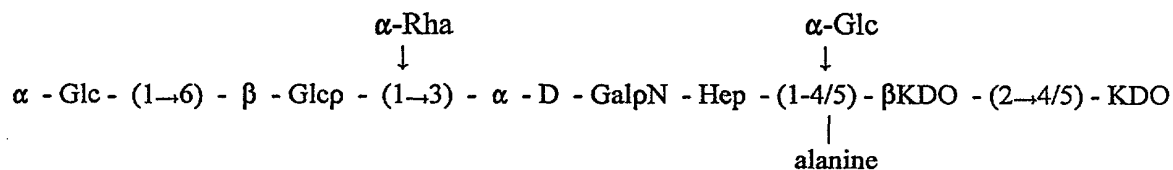
47. The method of claim 44, wherein the polysaccharide comprises



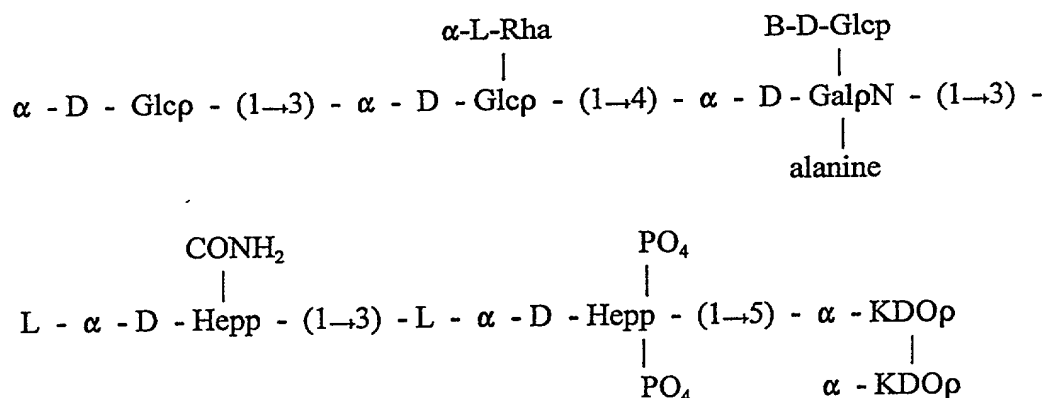
48. The method of claim 44, wherein the polysaccharide comprises



49. The method of claim 44, wherein the polysaccharide comprises



50. The method of claim 44, wherein the polysaccharide comprises



51. The method of claim 44, wherein the polysaccharide comprises a CFTR binding fragment of a lipopolysaccharide of *Pseudomonas aeruginosa*.

52. The method of claims 44-51 wherein the bioactive agent is covalently coupled to the polysaccharide.

53. The method of claims 44-51 wherein the bioactive agent is contained in a liposome comprising a lipid biocompatible with a human subject, and wherein the polysaccharide is covalently coupled to the lipid.

54. A composition of matter comprising  
a covalent conjugate of an anti-*Pseudomonas* drug and a cystic fibrosis transmembrane conductance regulator or a *Pseudomonas* lipopolysaccharide-binding fragment of a cystic fibrosis transmembrane conductance regulator.

55. The composition of claim 54 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 4 consecutive amino acids of SEQ ID NO 3.

56. The composition of claim 54 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 5 consecutive amino acids of SEQ ID NO 3.

57. The composition of claim 54 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 6 consecutive amino acids of SEQ ID NO 3.

58. The composition of claim 54 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 7 consecutive amino acids of SEQ ID NO 3.

59. The composition of claim 54 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 8 consecutive amino acids of SEQ ID NO 3.

60. The composition of claims 54-59 wherein the anti-*Pseudomonal* drug is an antibiotic.

61. The composition of claims 54-59 wherein the anti-*Pseudomonal* drug is selected from the group consisting of Amifloxacin; Amifloxacin Mesylate; Amikacin; Amikacin Sulfate; Aminosalicyclic acid; Aminosalicylate sodium; Azlocillin; Azlocillin Sodium; Bacampicillin Hydrochloride; Carbenicillin Disodium; Carbenicillin Indanyl Sodium; Carbenicillin Phenyl Sodium; Carbenicillin Potassium; Carumonam Sodium; Cefaclor; Cefadroxil; Cefamandole; Cefamandole Nafate; Cefamandole Sodium; Cefapareole; Cefatrizine; Cefazaflur Sodium; Cefazolin; Cefazolin Sodium; Cefbuperazone; Cefdinir; Cefepime; Cefepime Hydrochloride; Cefetecol; Cefixime; Cefmenoxime Hydrochloride; Cefmetazole; Cefmetazole Sodium; Cefonicid Monosodium; Cefonicid Sodium; Cefoperazone Sodium; Ceforanide; Cefotaxime Sodium; Cefotetan; Cefotetan Disodium; Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceftizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalixin; Cephalixin Hydrochloride;

Cephaloglycin; Cephaloridine; Cephalothin Sodium; Cephapirin Sodium; Cephadrine; Cetocycline Hydrochloride; Cetophenicol; Chlortetracycline Bisulfate ; Chlortetracycline Hydrochloride ; Ciprofloxacin; Ciprofloxacin Hydrochloride; Colistin Sulfate; Coumermycin; Coumermycin Sodium; Doxycycline; Doxycycline Calcium; Doxycycline Fosfatex; Doxycycline Hyclate; Droxacin Sodium; Enoxacin; Epicillin; Eptitetracycline Hydrochloride; Imipenem; Kanamycin Sulfate; Meclocycline; Minocycline; Minocycline Hydrochloride; Nafcillin Sodium; Norfloxacin; Ofloxacin; Oxytetracycline; Oxytetracycline Calcium; Piperacillin Sodium; Pirbenicillin Sodium; Tetracycline; Tetracycline Hydrochloride ; Tetracycline Phosphate Complex; Ticarcillin Cresyl Sodium; Ticarcillin Disodium; Ticarcillin Monosodium; Tobramycin; and Tobramycin Sulfate.

62. A polypeptide comprising an isolated *Pseudomonal* lipopolysaccharide-binding fragment of a cystic fibrosis transmembrane conductance regulator.

63. The composition of claim 62 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 4 consecutive amino acids of SEQ ID NO 3.

64. The composition of claim 62 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 5 consecutive amino acids of SEQ ID NO 3.

65. The composition of claim 62 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 6 consecutive amino acids of SEQ ID NO 3.

66. The composition of claim 62 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 7 consecutive amino acids of SEQ ID NO 3.

67. The composition of claim 62 wherein the *Pseudomonas* lipopolysaccharide-binding fragment comprises at least 8 consecutive amino acids of SEQ ID NO 3.



68. A method for targeting an anti-*Pseudomonas* drug to *Pseudomonas* comprising contacting the environment of the *Pseudomonas* with a compound of claims 54-61.

68. A method for targeting an anti-*Pseudomonas* drug to *Pseudomonas* comprising contacting the environment of the *Pseudomonas* with a compound of claims 54-61.

### Abstract

Methods and products for upregulating cystic fibrosis transmembrane conductance regulators are provided, including methods and products for the treatment of *P. aeruginosa* infection. The products include polysaccharides that interact with the cystic fibrosis transmembrane conductance regulator (CFTR). The polysaccharide compositions of the invention may be administered to a subject in order to enhance the uptake of *P. aeruginosa* into the epithelial cells of the subject. The invention also encompasses compositions comprising a lipopolysaccharide-binding region of a CFTR linked to an anti-*Pseudomonas* drug and methods of use of such compositions. Compositions and methods for gene therapy are also disclosed. The compositions include polysaccharides that bind to CFTR coupled to a gene delivery vehicle.

62014.1

## DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

## METHODS AND PRODUCTS FOR TREATING PSEUDOMONAS INFECTION

the specification of which

(check one) ☒ is attached hereto

☐ was filed on \_\_\_\_\_, as Application Ser. No. \_\_\_\_\_ amended on \_\_\_\_\_ (if applicable).

☐ was filed as a PCT international application No. \_\_\_\_\_, on \_\_\_\_\_ and was amended under PCT Article 19 on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign PCT Application(s) and any priority claims under 35 U.S.C. §119:			Priority Claimed	
(Number)	(Country if PCT so indicate)	(DD/MM/YY Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			YES	NO
(Number)	(Country)	(DD/MM/YY Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			YES	NO
(Number)	(Country)	(DD/MM/YY Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			YES	NO

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

_____ (Application Number)	_____ (filing date)
_____ (Application Number)	_____ (filing date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

_____ (Application Serial No.)	_____ (filing date)	_____ (status-patented, pending, abandoned)
_____ (Application Serial No.)	_____ (filing date)	_____ (status-patented, pending, abandoned)

PCT Applications designating the United States:

(PCT Appl. No.)	(U.S. Ser. No.)	(PCT filing date)	(status-patented,pending,abandoned)
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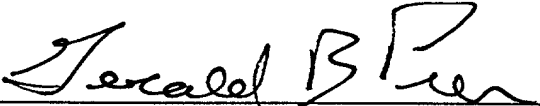
I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

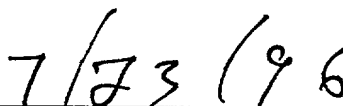
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

  
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